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FACTORS INFLUENCING THE ASEXUAL PERIODICITY OF AVIAN MALARIAS[1]

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INTRODUCTION

The mechanism underlying the length of the asexual cycle and the synchronism of reproduction is of basic importance in obtaining not only a better knowledge of the malaria parasites, but of the host reaction as well. The diversity of periodic rhythms among the malaria parasites, however, complicates the problem. Among the avian parasites alone, there are species or strains which have little or no periodicity. Others have cycles lasting 48 hours (Huff, 1935; Wolfson, 1936b), 36 hours (Gingrich, 1929), 30 hours (L. G. Taliaferro, 1925), 24 hours (L. G. Taliaferro, 1925; Huff and Bloom, 1935; Huff, 1937; Wolfson, 1936a, 1937), or 12 hours (Bovet, 1930). Some segment in the morning hours while others segment during the evening hours. W. H. Taliaferro (1932) suggested that the length of the asexual cycle of each malaria infection is largely determined by the genetical constitution of the parasite, but that the time of segmentation and the synchronism of reproduction are largely determined by the host. If only the host factor were involved, all parasites would have quotidian infections. If the host played no part, each strain might be expected to have a different type of infection. Most of the strains of malaria, however, have synchronous rhythms which are multiples of 24 hours.

If the part the host plays be largely of the nature of a consistent but cyclically changing culture medium, alteration of this culture medium should lead to a better knowledge of the factors controlling the synchronism and time of segmentation. Changes in temperature, light, feeding, host activity and rest were accordingly used in this study of the

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periodicity of the asexual rhythm. Of these, environmental temperature and the period of rest or sleep were measurably effective. It was demonstrated that light acted by compelling the host to activity—a result produced through the eyes as distinguished from a body surface effect. In the life cycle of the parasite the young trophozoite seemed to be the stage most susceptible to these influences. In the host the diurnal fluctuation of body temperature or a closely related phenomenon appeared to be responsible for the synchronism and time of appearance of asexual reproduction.

HISTORICAL REVIEW

Golgi first reported on the asexual cycles of the malaria parasites of man and related them to the familiar tertian and quartan fevers. In 1925, L. G. Taliaferro demonstrated that *Plasmodium cathemerium,* in the canary, regularly and synchronously reproduces every 24 hours with schizogony occurring largely from 6–9 P M. These original observations have been abundantly confirmed for *P. cathemerium* by Drensky and Hegner (1926), Hartman (1927), G. H. Boyd (1929a and b, and with Allen in 1934), Huff and Gambrell (1934), Lourie (1934) and by the work reported here. The work has been extended in recent years, especially by Huff and also Wolfson, to include studies of the periodicity of many of the growing list of bird malaria parasites inoculable into the canary.

This striking periodicity of reproduction naturally led to speculations as to its nature and mechanism. Is it an inherent property of the parasite or is it conditioned by the host? In 1928, L. G. Taliaferro showed that by *in vitro* refrigeration for 6 or 12 hours reproduction of *P. cathemerium* could be delayed by an interval equal to the time of refrigeration, but that it soon returned to normal when the refrigerated parasites were inoculated into a normal canary. Boyd (1929a) by reversing the light and dark schedule, obtained a reversal of the periodicity of the asexual reproduction of *P. cathemerium.* It is to be noted that the time of segmentation and not the length of the asexual cycle was changed. W. H. and L. G. Taliaferro (1934b) obtained a similar result with the quartan malaria parasite, *P. brasilianum.* The simian parasite, however, not only shifted its peaks of segmenting parasites, but also split into two broods, one segmenting 12 hours previous to and the other 12 hours subsequent to the original time of segmentation. Gambrell (1937), by a similar procedure, was able to reverse the time at which the peaks of gametocytes as well as of segmenting asexual parasites appeared. She used the "D" strain of *P. cathemerium* and the "R" strain of *P. relictum* var. *matutinum* in both of which the periodic production of gametocytes had been determined. In another paper Boyd (1929b) found that alter-

nate light and dark periods of 6, 8 or 16 hours produced no effect on the 24 hour periodicity, but that alternate light and dark periods of 14 or 20 hours disturbed the rhythm and that prolonged exposure to an experimental day and night of 14 hours each changed the periodicity from 24 to 28 hours. Gambrell (1937) found that, when canaries were subjected to alternate 24-hour periods of light and darkness, gametocyte production became asynchronous concomitantly with the loss of the periodic asexual rhythm. In 1933 Boyd seemed to eliminate host fatigue as a factor in influencing the reproductive activity of the parasite but suggested that "factors associated with host feeding seem to exert considerable influence upon the reproductive activity of this parasite." Huff and Gambrell (1934) found that races of *P. cathemerium* ("D" strain) not producing gametocytes did not show a periodic rhythm of asexual reproduction. Gambrell (1937) obtained from the "N" strain also a series of strains of parasites completely lacking in gametocytes and in periodicity of the asexual forms.

Other modifications of the synchronism or periodicity of the malaria parasite have been attempted. For example, L. G. Taliaferro (1925) reported that in *P. cathemerium,* in one case at least, the parasites of the first and second relapses did not show as high a degree of synchronism as in either the acute or chronic periods. Since adrenalin chloride was used to initiate the relapses we have a possible effect of this drug on the periodicity of the parasites. Boyd and Allen (1934) learned that quinine hydrochloride administered daily to infected canaries retarded the rate of growth of the parasite, influenced the adult size and delayed the time of the asexual cycle. Lourie, in 1934, in a more extensive publication on the mode of action of quinine independently came to similar conclusions. Wolfson (1936b) noted an apparent disturbance in the regularity of the periodicity of the avian tertian parasite (*P. circumflexum*) after a large amount of blood had been removed from the bird. In their extensive study of a simian malaria parasite, W. H. and L. G. Taliaferro (1932, 1934a, b and c) observed several modifications of the synchronism and length of the asexual cycle. They noted that if the parasites were refrigerated *in vitro* for several hours before injection into an uninfected monkey, or occasionally under ordinary manipulations, the first cycle took 4 days, and that both the synchronism and length of the asexual cycle of the parasites were disturbed occasionally during the crisis of the acute infections and also of the superinfection due to the immune response of the host. In all the above cases, however, when the abnormal factor was no longer acting (quinine, manipulation, refrigeration, etc.) the normal rhythm quickly returned although with the tertian and quartan parasites the peaks of segmentation might be a day or two later than anticipated.

MATERIALS AND METHODS

The female canary, *Serinus canarius,* was used throughout this work. One of the toe veins was used for all intravenous injections of parasites and blood for smears was taken from a leg vein. To study the parasites, blood smears were generally made at 2- or 4-hour intervals over a period of 72 (sometimes 24) hours and were air dried, fixed in absolute methyl alcohol and stained with Mac Neal's tetrachrome, prepared according to Gingrich's modification (1932).

The following three strains of *P. cathemerium* and one strain of *P. relictum* var. *matutinum* were used: "C" strain, *Plasmodium cathemerium,* isolated from a sparrow by Dr. Ernest Hartman in 1924, and used by L. G. Taliaferro (1925 and 1928) and G. H. Boyd (1929a and b, 1933 and 1934); "N" strain, derived from a single parasite of *P. cathemerium;* "D" strain, *P. cathemerium,* isolated from a sparrow by Dr. Hackett in Italy, shipped to Dr. W. Gingrich in mosquitoes and used by Huff (1934) for numerous infectivity experiments; "R" strain, *P. relictum* var. *matutinum,* isolated from a robin by Huff (1937).

The "C" "D" and "N" strains of *P. cathemerium* are similar except that the "D" and "N" strains occasionally tend to produce strains that lack gametocytes and periodicity. The periodicity is synchronous with the peak in segmentation occurring in the evening hours under animal room conditions or at the beginning of the dark period under more rigidly controlled conditions (see L. G. Taliaferro, 1925, and others for the "C" strain; Huff and Gambrell, 1934, and Gambrell, 1937, for the "D" and "N" strains). The periodicity of the "R" strain of *P. relictum* var. *matutinum* is extremely synchronous with the peak in segmentation occurring in the morning hours or at the beginning of the light period. Since the periodicity of this strain is so synchronous, it would have been particularly suitable for use in this work, but unfortunately infections were often very low grade and transitory.

To regulate the light and dark periods, a cabinet containing four light-tight compartments with separate light sources and time switches (Fig. 1) was used. Birds were placed in standard wire bird cages within a compartment. The illumination at the approximate center of these cages was measured by means of a Weston or Westinghouse light meter, a photoelectric cell with the ammeter graduated in foot candles of light. Control birds were always supplied with a light source equivalent to conditions better than the poorest in the animal room on a cloudy day. Where the light might have been of such an intensity as to interfere with the usual air temperature of a compartment, water filters were used.

The method used by W. H. and L. G. Taliaferro (1934a) to study periodicity was adopted in this work. It involves ascertaining the percentage of segmenters in a random sample of parasites on stained blood

films at close intervals for a sufficient time during the course of a malarial infection. Thus, in a strain showing periodicity, the percentage of segmenters will be none or very few for a certain number of hours and then will increase to a peak and decrease as sporulation occurs. The method is adequate provided it is modified to fit the characteristics of a given periodicity. For example, smears will need to be made more often to show a periodicity of 24 hours than to show one of 72 hours; a segmenter will need to be considered as a parasite with 5 or more nuclei if the completed segmenter forms very few merozoites; and care has to be taken to make blood films within the sporulation period. (In my

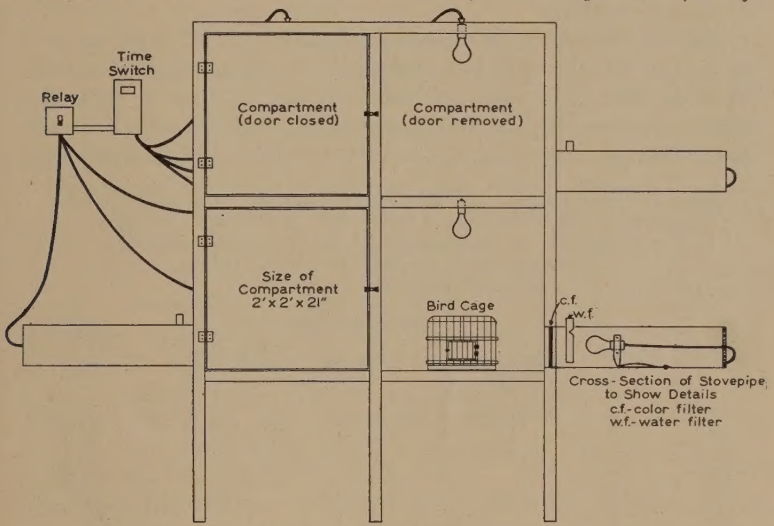

FIG. 1. Sketch of the light-tight cabinet used.

own work, I found that sharper higher peaks were obtained in *P. cathemerium* infections under ordinary conditions if the smears were made at 4, 6 and 8 P M rather than at 3, 5, 7 and 9 P M.) In this connection, it must be remembered that the peak of segmenters precedes the actual peak of schizogony by a few hours. The procedure which I found best for my work was to make smears at 2- or 4-hour intervals, over a consecutive period of 72 hours (sometimes less), to examine 200–300 parasites (50–100 in the few cases where parasites were scarce) on each smear and to consider a segmenter to be a parasite with 8 (in a few instances 5; see Fig. 6) or more nuclei.

The Effect of Light on Periodicity

To determine whether light influences periodicity and whether it acts through the eyes or through the body surface of the canary, the follow-

ing experiment was devised. Twenty-four canaries were placed in groups of 8 in three compartments of the light-tight cabinet under the following conditions. A 40-watt bulb furnished the illumination for the light periods in all compartments. Compartment A had continuous light with food constantly present. Compartment B had continuous light with food present during only 12 hours of each 24-hour period (10 A M–10 P M). Compartment C had 12-hour periods of light with food (10 P M–10 A M) alternating with 12-hour periods of darkness without food. These are essentially controls, but it is to be noted that their light (10 P M–10 A M) and dark (10 A M–10 P M) periods were a reversal of normal conditions.

On the 10th day of the experiment birds 1C and 2C in compartment A, 3C and 4C (this bird died prematurely) in compartment B and 5C–8C in compartment C were inoculated with the "C" strain. Three days afterwards, these birds were killed and their blood inoculated intravenously into the 16 other birds, as indicated in Table I. In the meantime,

TABLE I.—*Cap experiment of 18 days duration, showing the conditions of light, darkness, food and wearing of cap. From 15th through 18th day of experiment, i.e., beginning 2nd day after birds were infected, slides were made for 4 days at approximately 2-hourly intervals (See Figs. 2 and 3)*

Bird number	Group	Conditions in compartment			Infected on 13th day of Exper. from :
		Light during 18 days	Food during 18 days	Caps 10AM–10PM 5th to 18th day	
9C	A b	Continuous	Continuous	No	1C, 2C[1]
10C	" b	"	"	"	5C–8C[2]
15C	" c	"	"	Yes	1C, 2C
16C	" c	"	"	"	1C, 2C
17C	" c	"	"	"	5C–8C
18C	" c	"	"	"	5C–8C
11C	B b	"	10AM–10PM	No	3C[3]
12C	" b	"	"	"	5C–8C
19C	" c	"	"	Yes	3C
20C	" c	"	"	"	3C
21C	" c	"	"	"	5C–8C
22C	" c	"	"	"	5C–8C
13C	C b	10PM–10AM	10PM–10AM	No, but in dark 10AM–10PM	1C, 2C
14C	" b	"	"	"	5C–8C
23C	" c	"	"	"	3C
24C	" c	"	"	"	5C–8C

[1] 1C, 2C, pooled blood from 2 birds which had been kept in continuous light with continuous food for 13 days and which 3 days before being killed were inoculated with the "C" strain.

[2] 5C–8C, pooled blood from 4 birds which had been kept in light with food for 12 hours and dark without food for 12 hours for 13 days and which 3 days before being killed were inoculated with the "C" strain.

[3] 3C, blood from 1 bird which had been kept in continuous light with food 10 A M–10 P M for 13 days and which 3 days before it was killed was inoculated with the "C" strain.

on the 5th day of the experiment, four birds in compartment A (15C–18C) and four birds in compartment B (19C–22C) were made to wear caps in the interval 10 P M–10 A M each day thereafter until the end of the experiment whereas the remaining birds in compartment A (9C, 10C), in compartment B (11C, 12C) and in compartment C (13C, 14C,

23C, 24C) were left under the conditions existing in their special compartments. The caps were made of black cloth with a beak slit for unhampered breathing and were fastened at the back of the head by a purse string arrangement. Although a bird struggled for a time after a cap was put on, it suffered no undue inconvenience and sooner or later settled to rest and remained so until disturbed. Smears of birds 9C through 24C were begun on the 15th day of the experiment, the parasites in the interim becoming adjusted to the conditions of their hosts. The infections in these birds were very heavy and of short duration with two-thirds of the birds having a definite number crisis during the period when smears were being made.

Figs. 2 and 3 show the results obtained in this experiment on the percentage of parasites occurring with 5 or more nuclei. The birds in the first 3 graphs of Fig. 2 were inoculated from birds 1C and 2C whose infection showed a somewhat disrupted periodicity due to the continuous light to which the birds were subjected. Bird 9C (not capped) exhibits no asexual periodicity of its malaria parasites, whereas bird 16C (capped) shows a definite asexual periodicity with segmentation peaks occurring at the end of the period when no cap was worn. Bird 15C which was also capped died prematurely. Bird 13C under control conditions, shows a slight asexual periodicity, but apparently this bird was unable during the course of the experiment to resolve the parasites into a brood segmenting with a clear-cut periodic rhythm. The birds in the last 3 graphs of Fig. 2 were inoculated from bird 3C whose infection showed a slightly disrupted periodicity. Bird 11C shows a slight periodicity, whereas the time of peak segmentation differs by 12 hours in birds 19C and 23C. Such a condition indicates that the periodicity of the parasites in each of these birds had become readjusted to sporulate at the beginning of the dark period and was, therefore, behaving normally with respect to light and dark. The birds in Fig. 3 received parasites from 5C–8C (normal periodicity) and showed a relatively high percentage of segmenters at the beginning of their infections. In birds 14C and 24C, under control conditions, the periodicity is maintained with the peaks appearing at the beginning of the period of darkness. In bird 10C, the periodicity is progressively lost. Bird 12C shows a gradual reduction in peak magnitude and a progressive shift of peak time from 10 A M to 6 P M near the end of the period of feeding. Birds 17C, 18C, 21C and 22C, which wore caps 10 P M–10 A M, all show a definite shift of segmentation from the 10 A M hour (of the injected parasites) toward the 10 P M hour. Although the segmenters were never present in large numbers, the constant direction of the shift of the peak time, the similar response in all four birds, and the presence of a periodicity seem to confirm the opinion that the parasites in these birds were arranging themselves into a cycle

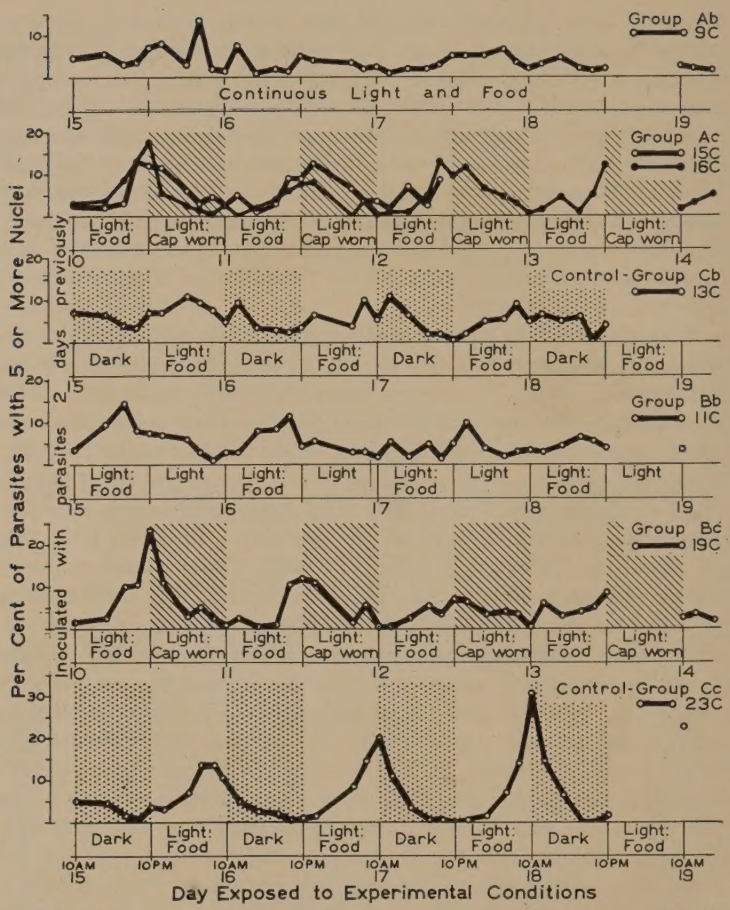

FIG. 2. The effect of various host conditions on the asexual reproductive rhythm of the "C" strain of *P. cathemerium*. Stippled areas indicate the host period of darkness, cross-hatched areas the period (relative darkness) when certain canaries wore caps.

of synchronous reproduction with the peak time at the beginning of the period in which they wore caps.

In summary, all of the capped birds (15C, 16C, 17C, 18C, 19C, 21C and 22C) responded in the same way regardless of the other conditions to which they were exposed or the source of the parasites used for inoculation. In some birds the time when segmenting parasites most frequently appeared definitely shifted, in others synchronicity was reconstructed from a condition in which none existed. In every case as the

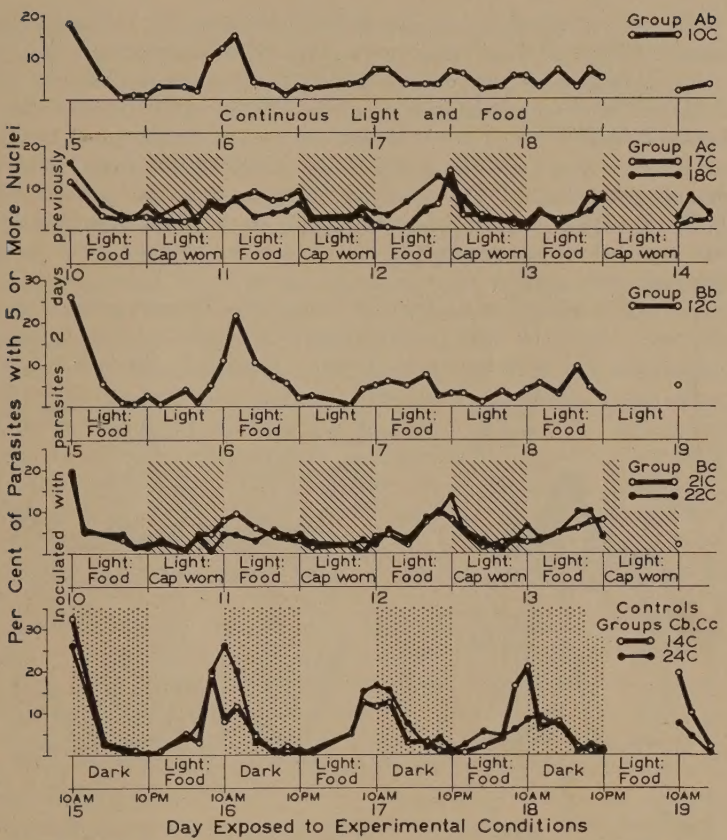

FIG. 3. The effect of various host conditions on the asexual reproductive rhythm of the "C" strain of *P. cathemerium*. Stippled areas indicate the host period of darkness, cross-hatched areas the period (relative darkness) when certain canaries wore caps.

infection progressed, the time of peak segmentation occurred near the beginning of the period when caps were worn. The influence of light on the general body surface seems ruled out as a factor in periodicity. The part played by the eyes in this experiment may be through a direct effect of light or through an indirect effect on the ability to feed or to rest.

The Effect of Environmental Temperature on Periodicity

Because the periodicity of the asexual cycle of avian malaria was found to be disturbed during a heat wave in the summer of 1934, some experiments on temperature changes were carried out.

In the first experiment, groups of birds were placed in the light-tight cabinet. Three of these groups were subjected to a temperature of 37° C by means of a hot plate during the dark foodless period (10 A M–10 P M) and were subjected to a temperature of between 24° C–29° C during the light-food period (10 P M–10 A M). The control group D was subjected to a temperature of 24° C–29° C during the dark foodless periods (10 A M–10 P M) as well as during the light-food periods (10 P M–10 A M). (It will be noted that all of the birds, including the bird from which the infection of "N" strain was obtained, were on a light and dark schedule the opposite of normal.) Of these groups, A and B were infected 3 and 1 day later, respectively, whereas group C was infected 3 days later with parasites from 1N which had already been subjected to high temperature for 4 days. Judging by the time, height

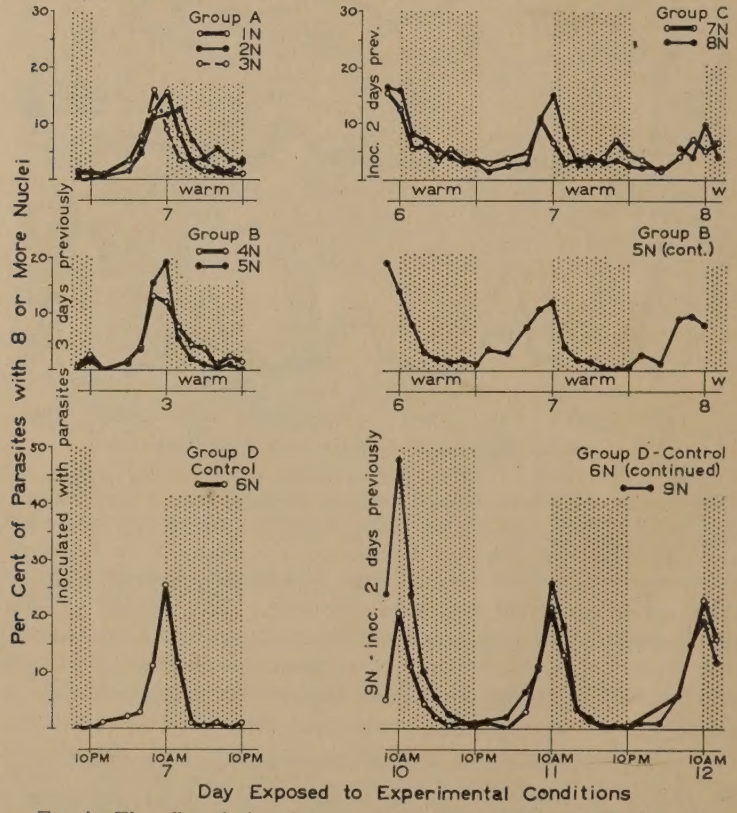

FIG. 4. The effect during the canary's period of darkness of high environmental temperatures on the asexual reproductive rhythm of the "N" strain of *P. cathemerium*. Darkness represented by stippled areas.

and spread of the peaks and the number of segmenters found in the periods between the peaks in groups A, B and C as compared with birds 6N and 9N in control group D in Fig. 4, the high temperature produces a disturbance in the periodic rhythm of segmentation of the parasites which is commensurate with the length of exposure. Thus, the parasites in birds 4N and 5N of group B which were subjected to high temperature for only a short time (2 days) before observations were made, show less disturbance in the cycle than those in either 1N, 2N and 3N of group A, the birds of which had been subjected to high temperature for a longer period (7 days) or those in 7N or 8N of group C, the birds of which and the parasites of which had both been subjected to high temperature for longer periods (6 days).

In the second experiment four groups of birds were subjected to the "R" strain and to more extreme temperature conditions which were made possible by an incubator and ice chest. The temperatures in the lighted half of the incubator were somewhat higher (31–43° C) than in the darkened half (31–38° C). The temperature in the ice chest varied from 14.4–20° C, but usually ranged around 15° C. Food was present during the light period for all birds. Birds 1R and 2R in group A were subjected to high temperature during the light period, and to cold during the dark period, and the procedure was reversed for birds 3R and 4R in group B. Birds 5R and 6R in group D were subjected to continuous high temperature whereas 7R and 8R in group E were subjected to continuous cold. The control bird 9R in group C was subjected to continuous normal room temperature. The "R" strain of parasites used for inoculation was originally obtained by pooling the blood from two birds which differed in day and night schedules and whose parasites, therefore, segmented at different times, so that the power to readjust the periodic rhythm could be studied. In birds under normal conditions, segmentation quickly reverts to a synchronous periodicity (see 9R, Fig. 5). These parasites were inoculated into one of the foregoing groups after they had been for 2 days in a cage mate. Thus, they had a sojourn in a bird under similar experimental conditions before they were injected into the test bird.

Although the infections in this series were low grade, a comparison of the per cent of parasites with 8 or more nuclei between the various groups revealed that the usual synchronism of the asexual cycle is disturbed by periodic changes in temperature (groups A and B) and also by continuous high temperature (Group D) or continuous cold (Group E). Except in bird 2R, however, the synchronicity was not affected to the degree that it was in the "N" strain (Fig. 4).

In a third experiment with the "D" strain performed in a manner similar to the second experiment in this series, the greatest deviation from

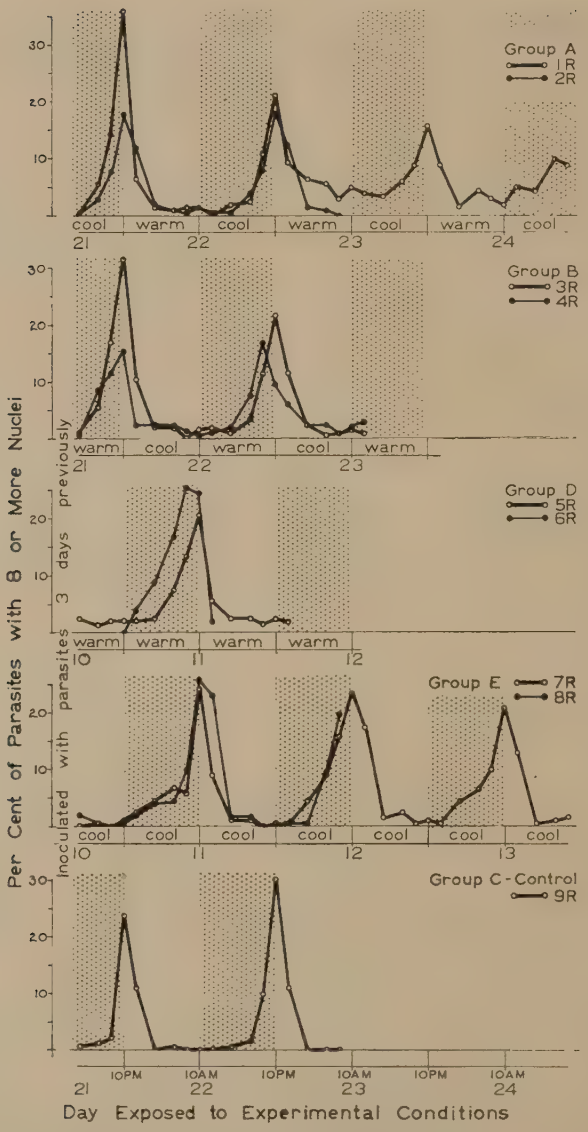

FIG. 5. The effect produced on the asexual reproductive rhythm of the "R" strain of *P. relictum* var. *matutinum* by exposing the canary host to unusual combinations of environmental temperatures. The stippled areas represent the period of darkness.

the control was obtained with group B birds subjected to incubator temperatures during the period of darkness and to ice chest temperatures during the light period while the other groups exhibited synchronous reproduction but with altered time in the peak of segmentation.

These three experiments seem to indicate that high environmental temperatures alternating with room or ice chest temperatures caused a significant disturbance in the asexual periodicity of these avian malarias. Moreover, since the most significant deviation in the cycle (see especially Fig. 4, 7N and Fig. 5, 2R) occurred when the temperature was high during the young trophozoite stage, it would seem that this portion of the life history of the parasite is more susceptible to increased atmospheric temperature surrounding the host than other portions. Whether the effect is produced directly on the growth rate of the parasite or indirectly through some other process or processes is not evident from the results obtained. Continuous high or cold temperatures failed to produce as pronounced results although some disturbance is noted in 5R and 6R. Until further data are obtained we assume that normal conditions of body temperature can be more nearly approximated in constant heat or cold than in alternations of the two.

The Effect of Activity and Feeding on Periodicity

The importance of feeding (with its concomitant muscular activity and resultant digestive and assimilative phenomena) and of rest in the control of the reproductive rhythm of the parasites was tested by alternating 12-hour periods of high and low intensity illumination and adjusting the time of feeding so that some birds (subgroup "a") were allowed to feed only during the period of higher intensity light while others (subgroup "b") were allowed to feed only during the hours of lower intensity light. The intensity of light was varied as follows: In compartment A which represented the greatest range of light conditions, the center of the cage received 105 foot-candles of light from 100-watt bulb 10 A M–10 P M and 1.5 foot-candles of light from a 7½-watt bulb from 10 P M–10 A M. In compartment B, the alternate intensities for the same time intervals were 41 foot-candles of light from a 60-watt bulb and 4.5 foot-candles of light from a 15-watt bulb. Similarly, in compartment C, the alternate intensities were 20 foot-candles of light from a 40-watt bulb and 7.3 foot-candles of light from a 25-watt bulb. The birds were adapted to the experimental conditions for 14 days before infection except in subgroups Ab and Ca (Fig. 6) where the birds originally intended for the experiment died prematurely and others had to be substituted. The "N" strain for this experiment came from the pooled blood of 2 birds, one of which had been kept under ordinary animal room conditions with normal periods of daylight and darkness and the other

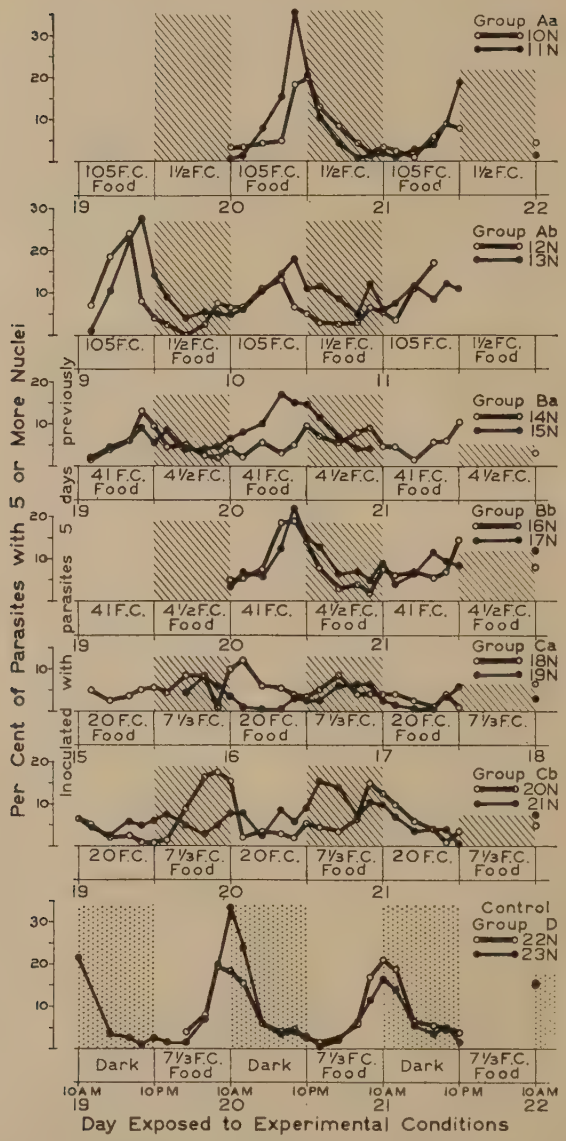

FIG. 6. The effect on the asexual reproductive rhythm of the "N" strain of *P. cathemerium* of exposure of the canary to alternate periods of different intensity illumination. Cross-hatched areas represent period of lower intensity illumination or relative darkness. The stippled areas represent the period of darkness for the control birds.

of which had been kept in the light-tight cabinet on a reversed schedule. The 12 experimental birds and 2 controls were inoculated intramuscularly and 5 days later blood smears were made at frequent intervals to cover at least about 2 complete asexual cycles of the parasite. These data will be found in Fig. 6.

The periodicity obtained in the control birds 22N and 23N is slightly less regular than that obtained in the temperature experiment with this strain of parasites, but the peaks of segmentation occur, as would be expected, at or near the beginning of the period of darkness. The birds in group Aa, (10N, 11N) which were subjected to 105 foot-candles with food and 1.5 foot-candles without food show an asexual periodicity almost as marked as in the controls with the peaks appearing at or near the beginning of the period of lower light intensity or relative darkness. The birds in subgroup Ab (12N, 13N) which were fed during the period of lower intensity light also show definite peaks in segmentation at the beginning of the period of lower light intensity, but the peaks are not so sharp. The birds in group Ba (14N, 15N) under more nearly equal light intensities consisting of alternate periods of 41 foot-candles with food and 4.5 foot-candles without food, and the birds in group Bb (16N, 17N) which were fed during the low light intensity also show a major segmentation near the beginning of the period of lower light intensity, which, however, is not nearly so marked as that in the controls. Thus, the data for these 4 groups indicate that the period of lower intensity of light exerts a greater influence than the time of feeding. The birds in compartment C, where the light intensity alternated from 20 to 7.3 foot-candles, show results more nearly like those reported for continuous light. In subgroup Ca (18N, 19N) there is almost a complete disruption of the synchronism, with segmenters found on nearly every slide and the peaks with one exception less than 10 per cent. The birds in subgroup Cb (20N, 21N) also show a disruption of the customary periodicity of the malaria parasites. Bird 20N shows well-defined peaks of segmentation occurring at 8 A M on successive days near the *end of the period of food and lower intensity light.* Peaks occur in 21N at almost 12-hour intervals, possibly reflecting the two broods of parasites inoculated.

During the course of this experiment as blood smears were made, the number of sleeping birds in each cage was recorded in order to obtain an idea of the activity of the birds. In compartment A where the light intensity differed most, the birds slept only during the period of low intensity light although birds of subgroup "b" also had to feed in this period. If the period of rest is taken as our focal point, regardless of whether or not it was interspersed with feeding, we find the peak of segmentation related to the rest period, or probably, as Boughton (1933)

suggests for coccidia, to a previous rest period. Birds in group B showed similar periods of rest (an occasional bird was found asleep during the period of high intensity illumination) and again the data point to a relationship with the period of rest or sleep. Birds in group C, where the light was more nearly continuous, were found asleep at various times, but predominantly during the period when no food was allowed them regardless of the light intensity. The period of rest, therefore, in these birds became related to the period of feeding. In no cage did as many as 100 per cent of the observations show birds asleep. During the period of study blood smears were being made frequently during the day. This constant disturbing factor, especially in the presence of continuous light, must certainly have produced this effect, at least in the birds in subgroups "a." In birds of subgroups "b" this disturbing factor would also act but the necessity for feeding in the low light period would of itself decrease the percentages of sleep observations in this period.

A second experiment, using the "C' strain of *P. cathemerium* performed under the same conditions and procedure, gave confirmatory results.

Since the "R" strain of *P. relictum* var. *matutinum* shows such a markedly periodic and synchronous asexual reproductive activity and since it sporulates normally at the beginning of the light period instead of at the beginning of the dark period as does *P. cathemerium,* it was tested under the same conditions and procedure outlined above. The infections were low grade and transitory, typical of the "R" strain, and in some cases only enough parasites to make one complete cycle could be obtained, but the results, shown in Fig. 7, are surprisingly similar to those met with in the "N" strain.

In these experiments where light and dark are minimized and activity and feeding are accentuated two broods of parasites were injected into each bird, one of which segmented in the evening hours and the other in the morning hours. In most of the birds that brood became dominant whose sporulation time had the same relation to the rest period that the sporulation time in normal birds has to dark and light. This is extremely interesting in view of that fact that the brood of the "N" strain dominated whose sporulation took place at the beginning of the rest period (10N, 11N, 16N, 17N, 19N, 20N) and the brood of the "R" strain dominated whose sporulation took place at the beginning of the active period (10R, 11R, 13R, 14R, 17R, 18R). In a few birds, however, both broods tended to be maintained (21N, 15R), and in a few others sporulation of the two broods seemed to shift to an intermediate time (12N, 13N, 14N, 15N, 18N, 12R, 16R). These data are consistent in indicating that the rest period is far more important than feeding in the periodic cycle of reproduction and that feeding only becomes important when it

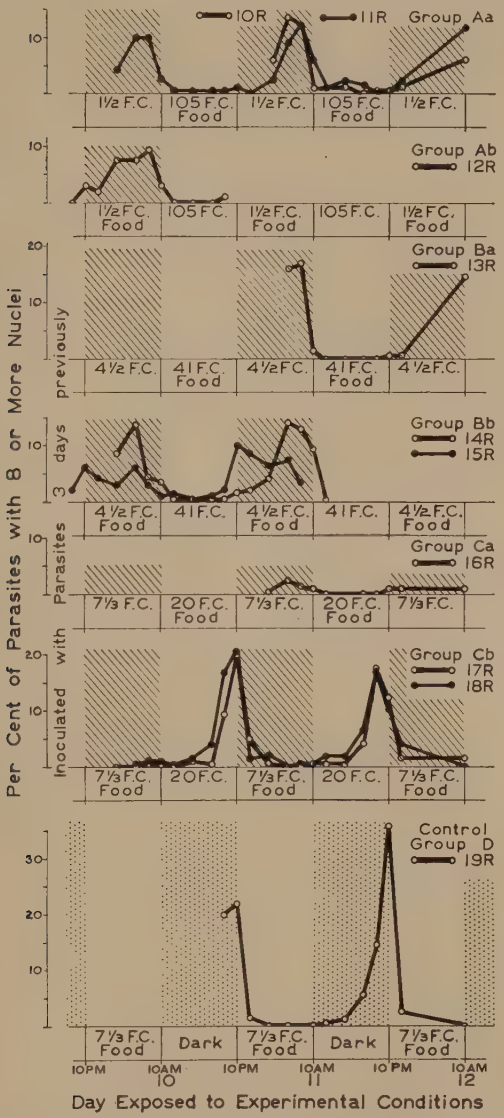

FIG. 7. The effect on the asexual reproductive rhythm of the "R" strain of *P. relictum* var. *matutinum* of exposure of the canary to alternate periods of illumination of different intensity.

interrupts or conflicts with the rest period. The next experiment bears out this conclusion.

In this experiment, 2 control and 2 experimental birds were exposed to light 11 P M–9 A M. During this time the control birds were allowed to feed at will, but the experimental birds were given no food (water was present) except in the last hour of the light period (8 A M–9 A M). At 9 A M both groups of birds were put in the dark except at intervals (roughly from 11:00 to 11:15 A M, 1:30 to 1:45 P M, 4:30 to 4:45 P M and 9:55 to 10:10 P M). During these light intervals, only the experimental birds were allowed to feed. After 12 days of acclimatization, the birds were inoculated with the "C" strain from two birds, and 4 days later blood smears were made and studied. Since the parasites in both groups showed cycles of reproduction entirely similar to that usually seen with the "C" strain of parasites, no graphs of the results are presented. The peak of segmentation occurred at the end of the light period regardless of the time food was taken.

DISCUSSION

A study of the periodicity in malaria is complicated by several variable factors. In quartan simian malaria, W. H. and L. G. Taliaferro (1934a) have shown that the crisis of the malaria infection may at times delay the asexual cycle by as much as a whole day. Boyd and Allen (1934) have also shown that as the acute infection proceeds the mean size of the segmenting parasite and the number of merozoites produced by it both diminish as the parasite number increases until the crisis is reached and then slowly return to their former level. Therefore, we have attempted to study only the acute rise of the infections herein presented. That this was not possible in every case is due in part to differences in the type of malaria infection produced by the various species, to the route of inoculation and to the dosage. Furthermore, to minimize loss of blood and disturbance to the bird, very small smears were made as quickly and gently as possible.

Boyd (1933) believed that host feeding is an important factor in periodicity, but the time of sleep may be the important factor since he did not separate the two. In all cases except one he used continuous light. According to our observations, however, birds do not tend to rest well in the light, especially when blood smears are being made at frequent intervals. In one case where continuous light was not present, his birds were force-fed during the dark period.

It is known from Szymanski's work (1920) that the canary is an "optical" animal, restricting its activity to the hours of exposure to light. It is also known that birds have a diurnal temperature cycle which is high during the light hours and lowest during the dark or rest period (L. G. Simpson and Galbraith, 1905, or Kendleigh and Baldwin, 1928).

This has been confirmed for the canary by Huff (unpublished). The synchronism of asexual reproduction of avian malaria may depend on the body temperature of the host. Huff has found that canary temperatures may vary 6° C between the daily maximum and the daily minimum. Critical temperatures for reproduction are a well-known phenomenon. For example, the oyster, *Ostrea virginica,* will not spawn in Barnegat Bay, N. J., until the water temperature has reached 20° C. Even this temperature is more effective if reached suddenly than when very gradually (Nelson, 1928). If we assume that some stage in the avian reproductive cycle or some phase of the parasite's metabolism has a critical temperature that is reached once daily in the canary, we need only further assume that a definite rest period is necessary to obtain a temperature differential great enough for this effect to be expressed. On this basis the results presented here all fall into line. Any disturbance of the rest period could then alter or shift the time of peak segmentation. Environmental temperatures surrounding a somewhat poikilothermic animal (the canary) could also modify the usual cycle of reproduction. Other factors, as time of feeding, when not interfering with the rest period would not affect the cycle. Light itself would exert a direct effect only in stimulating activity in the host. There seems to be, however, a special relationship in the temperature experiments. When high environmental temperatures were alternated with low temperatures, marked disturbance of the synchronism was observed only when the high temperatures were present during a certain stage of the parasite (young trophozoite). When the high atmospheric temperatures occurred during the other half-day no such marked disturbance of the cycle was obtained.

Can species differences also fit into such an hypothesis? For example, the "R" strain segments at a time which is antipodal to that seen in the "C" strain. We can assume either that altogether different factors act on different stages of the life cycle, that different factors act on a similar stage in the reproductive cycle or that the same factor acts on the same stage of the parasite, but that the critical level of action is different. The last alternative would furnish the situation explainable on the basis of body temperature. In support of this contention we can refer to the temperature experiments in which the most marked deviations of reproductive rhythm occurred (in those birds infected with either "R" or "C" strain parasites) when high environmental temperatures prevailed during that stage of the parasite cycle when almost all the forms were young trophozoites. Both Boyd (malaria) and Boughton, Atchley and Eskridge, 1935 (coccidia) found that lengthening the bird's day to an artificial day of 28 or 29 hours respectively resulted in a lengthening of the time between the peaks of segmenters or oöcysts to correspond to the artificial days. They also noted, however, that the

time of the peak had shifted from the evening hours to the morning hours. Since we do not know the details of the host response to these conditions, we can only postulate that either some of the parasites in the clone have slightly different critical levels which are favored or inhibited by the new conditions or that with the loss of the original critical factors another set comes into play. All other deviations, such as parasites with 30-, 36- and 48-hour cycles of reproduction can be explained on a genetical basis with or without a synchronizing factor or by assuming that the canary is an abnormal host. There is no reason for assuming that every strain of malaria should have an asexual periodicity. A counterpart is observed in filarial studies where both synchronous and asynchronous strains exist. In filariasis, the presence or absence of a cycle has been related to the biting habits of the insect vector. In malaria, since the presence of mature gametocytes shows a periodicity which is related to the asexual rhythm (Gambrell, 1937) we might suspect that the biting habits of the natural vectors of the various species of avian plasmodia are related to these periodic phenomena. The specific natural vectors of the avian malarias are not known, however.

It is true that the proof for this hypothesis is not yet conclusive, but it does make a working theory which seems at present to explain all the known major modifications of the periodicity of avian malaria. Only further study will demonstrate its true value. One point, however, requires emphasis. The young trophozoite seems to be the stage most susceptible to experimental influences and probably plays the important role in orienting the synchronous rhythm. There is also the possibility that what we have designated as critical body temperature may actually be some other physical state or physiological process which occurs simultaneously and synchronously with the changes in temperature and which is likewise affected by changes in environmental temperature, period of rest, etc., as we have assumed for body temperature.

SUMMARY

1. A study of the experimental modification of the asexual periodicity of avian malaria was conducted on three strains of *P. cathemerium* ("C," "D" and "N") and on one strain of *P. relictum* var. *matutinum* ("R"). One of the strains ("N") was obtained by single-parasite isolation.

2. The synchronous periodicity of reproduction of avian malaria is affected by alternate 12-hour periods of high and low temperature.

3. It is affected by light itself—active through the eyes and not through the body surface—if of sufficient intensity to cause the host to be active.

4. As a corollary to (2), it is associated with rest or sleep which seem to be important orienting factors.

5. It is not affected by host feeding of the type devised in these experiments.

6. It is considered possible that the host may furnish a set of critical temperature conditions which orients the time of segmentation. In such an hypothesis, the period of rest becomes important in that it makes possible the temperature differential necessary for the appearance of the critical value.

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OBSERVATIONS ON A NATURAL INFECTION WITH *STRONGYLOIDES* IN THE DOG

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In March, 1938, we obtained a small, male hound puppy about five weeks old from the animal farm of the Harvard Medical School. This dog had been delivered to the farm the day before it was received at the laboratory. It had been purchased from a local dealer and originated from near Attleboro, Massachusetts. No further history could be obtained. It was a thin, undernourished animal and was suffering from a severe dysentery. A microscopic examination of the feces revealed the presence of hookworm eggs, *Toxocara canis* eggs, eggs of *Trichuris vulpis* and numerous rhabditiform larvae of *Strongyloides*. Hookworms, ascarids and whipworms are frequent parasites of local dogs and their presence here was of no particular interest. The finding of *Strongyloides* larvae, however, was of considerable interest not only because of its occurrence in a region where human strongyloidosis is rare and of foreign origin, but was also, as far as we were aware, the first natural infection with *Strongyloides* in the dog noted outside the Orient. From a review of the literature it appears that only two such infections in the dog have been recorded heretofore, the first of which was observed in China by Fülleborn (1914) and the second in India by Ware (1923).

The feces of this dog were rich in *Strongyloides* larvae and rich yields of filariform larvae were constantly obtained from cultured feces until early in April. At this time, during the customary examination of a fecal smear preparatory to using feces in culture, it was apparent that the larvae were much fewer than previously. The feces collected on the following days yielded the same results, although the mushy, blood-streaked stools indicated that the dog still suffered a slight dysentery. Almost overnight, what had been a rich source of material had become nearly barren. On May 5th we made an attempt to increase the degree of infection. Over 2000 infective larvae obtained from cultures of his feces were applied to his inguinal region. The dog was held until the fluid transporting the larvae had dried. For the next 13 days the number of larvae in fresh stools remained approximately the same as earlier noted but a progressive and rapid decrease began on May 18th. On May 31st a second attempt was made to increase the degree of infection. Over 3000 infective larvae were applied to the inguinal region. Even so, the results of subsequent culture and direct smear examination were almost

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entirely negative. From June 11th to June 28th all examinations were negative for *Strongyloides* although throughout this entire time the numbers of *Toxocara, Trichuris* and hookworm eggs showed no significant change. On June 28th the dog was sacrificed. At autopsy, adult specimens of *Ancylostoma caninum, Trichuris vulpis* and *Toxocara canis* were obtained from the small intestine, but although a thorough search for *Strongyloides* was made by direct examination of tissues (lungs, trachea, gall bladder and intestines) and by Baermann extraction of them, no evidence of its presence was revealed. It would appear that recovery had been complete and that its course was similar to that observed in dogs and cats experimentally infected with *Strongyloides stercoralis* from man (Fülleborn, 1914; Sandground, 1928). The larvae given to this dog on May 5th were obtained directly from cultures of its own feces, but those given on May 31st were obtained from the culture of feces from a second dog experimentally infected with the natural strain on the off chance that by passage through a second animal their infectivity for the first dog might thereby be increased.

We readily infected other dogs, both young and old, with larvae obtained from cultures of the naturally infected dog's feces. Rhabditiform larvae appeared in the feces of the experimental dogs from 7 to 11 days after infection. The course of the infection in these dogs was similar to that of the natural infection.

We failed entirely to produce demonstrable infection in 4 guinea pigs, 2 young cats and in an adult volunteer. We obtained no evidence that the parasite was infective for monkeys (*Macaca mulatta*). In our attempts to transmit the parasite to the above laboratory animals from 2000 to 5000 infective larvae were applied to shaved or clipped areas. Only 20 infective larvae were applied to the skin of the volunteer. It is possible that too few larvae were employed in this attempt to infect man and the failure of this passage is, therefore, inconclusive as to the identity of the species of *Strongyloides* involved. However, considering how easily rats have been infected with a single larva of *S. ratti* (Graham, 1936–38) it would appear that the number of larvae we employed in this experiment was sufficient to establish the infection if the parasite and host had been compatible. It would appear, therefore, from these limited observations, that, although *Strongyloides* of human origin can be readily transmitted to dogs and cats under laboratory conditions, the species occurring naturally in dogs probably is distinct and should be identified as *Strongyloides canis* Brumpt, 1922.

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THE LIFE HISTORY OF *HYMENOLEPIS EXIGUA*, A CESTODE OF POULTRY IN HAWAII*

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Hymenolepis exigua, a tapeworm parasite in the intestine of chickens, was first described by Yoshida in 1908 (English description, 1910) and has been reported to occur in Japan and Formosa. In 1936 the senior author reported the finding of this parasite in Hawaii and pointed out that the amphipod *Orchestia platensis* served as its intermediate host. Outside of the above report, no literature was available on the complete life cycle of this parasite. The present paper gives an account of the development of the parasite in the intermediate and final hosts and observations on invertebrates, other than amphipods, that may serve as intermediate hosts.

METHOD OF PROCEDURE

In experiments involving the development of the parasite in the amphipods, a layer of soil was placed on the bottom of each of several wide-mouth bottles, about 3 cm in diameter and 5 cm tall. The soil was slightly dampened and on it were placed uterine sacs containing eggs of the parasite which had been recovered from feces of experimentally infected chickens. Following this, about five amphipods, obtained from areas where no tapeworm infection had been noted, were placed in each bottle in order that they might ingest the eggs. The mouths of the bottles were covered with muslin to keep the soil from drying rapidly and to prevent escape of the amphipods. After the amphipods had been exposed for 48 hours, they were confined in bottles containing soil free from tapeworm eggs for various periods at room temperature (78° to 82° F) before they were dissected to determine the development of the larvae. The above method was used in exposing other invertebrates, except houseflies, reported in this paper, to infection by this parasite. In exposing houseflies to infection, the larvae of the fly were placed in bottles containing a small amount of poultry feces on which had been placed uterine sacs of the tapeworm.

In experiments involving the development of *H. exigua* in the final host (chicken), cysticercoids of this parasite obtained from naturally infected amphipods were fed to week-old laboratory-raised chicks. At the end of selected intervals, some of the chicks were killed to determine

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the size and location of the parasites; other chicks were kept to determine when eggs of the parasite first appeared in the droppings.

DEVELOPMENT OF LARVA IN THE INTERMEDIATE HOST

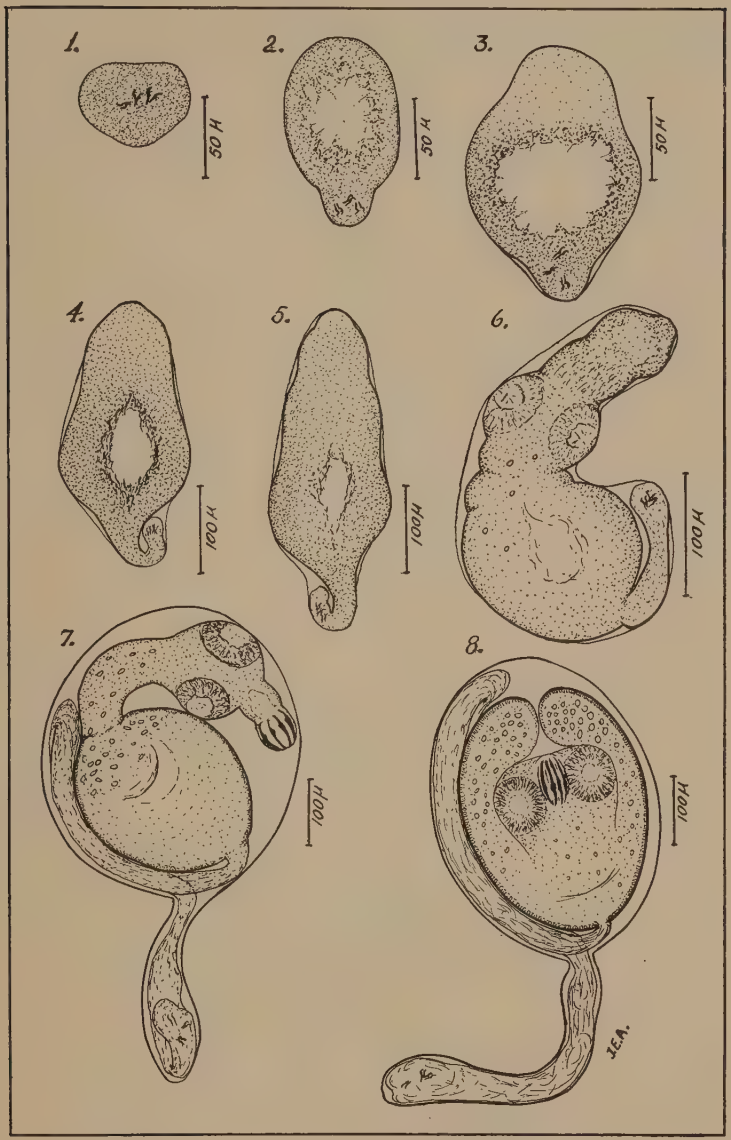
Amphipods dissected about 48 hours after exposure to infection contained in their body cavities many hexacanth larvae about 50 to 90 μ in diameter. Larvae measuring from about 50 to 60 μ showed more motility than larger ones and were in the "solid ball stage" (Fig. 1). Others, measuring about 67 to 90 μ, showed a central irregular cavity which appeared to have formed as a result of disintegration of the centrally located cells and gave the entire structure the appearance of a hollow ball.

Larval cestodes recovered from amphipods 5 days after infection were somewhat elongated, about 111 to 163 μ long by 74 to 105 μ wide, and with a central cavity (Figs. 2 and 3). The six embryonal hooks were located at the one end of the organism, which represented the anlage of the "tail." The larvae, at this time, were found to be surrounded by a thin transparent membrane (Fig. 3). The larvae which were largest in size showed, opposite to the tail, a less granular end, which from observations in later stages, gave rise to the neck and head of the organism.

Larvae secured from amphipods 6 days after infection were about 300 to 330 μ long and 104 to 120 μ wide, and had slender tails bent toward their bodies (Figs. 4 and 5). The anterior ends of the larvae were taking the form of a scolex. A more definite differentiation into the 4 parts of the body—head, neck, bladder and tail—was noted in larvae from an amphipod 7 to 8 days after infection (Fig. 6).

Cysticercoids from amphipods dissected 10 days after infection appeared to be fully developed, but as yet the heads were not invaginated (Fig. 7). The writers were not able to produce infection when such evaginated cysticercoids were fed to chicks. This observation confirms the report of Horsfall and Jones (1937) who noted that evaginated cysticercoids of *Choanotaenia infundibulum,* although they appeared completely formed, failed to infect primary hosts.

Larval cestodes recovered 12 days after infection showed invagination of the neck and scolex into the bladder (Fig. 8). This apparently terminated the development of the larva in the intermediate host since they produced an infection when fed to chicks. As reported previously by the senior author (1936), mature cysticercoids are 290 to 330 μ long and 267 to 282 μ wide. The rostellum of each is provided with 10 hooks about 45 μ long. The tail is usually long and partially coiled on itself. It also possesses near the posterior extremity the six embryonal hooklets. The tail is attached to the posterior portion of the bladder, but occa-



Figs. 1–8. *Hymenolepis exigua* larvae from the body cavity of experimentally infected amphipods. Fig. 1. Larva 2 days after infection. Figs. 2 and 3. Larvae 5 days after infection. Figs. 4 and 5. Larvae 6 days after infection. Fig. 6. Larva 8 days after infection. Fig. 7. Larva 10 days after infection. Fig. 8. Infective cysticercoid, 12 days after infection.

sionally it becomes separated. The whole larva, throughout most of its development within the intermediate host, is enveloped by the external membrane.

In addition to the feeding of *H. exigua* eggs to amphipods, attempts were made to experimentally infect sow-bugs (*Porcellio laevis*), beetles (*Tenebrioides nana*), houseflies (*Musca* sp.), and undetermined species of earthworms, but the results were negative. Evidence that these invertebrates had actually fed on the eggs was indicated by the absence or reduction in the number of uterine sacs placed in the bottles, and also by finding uterine sacs partly broken. The dissection of 100 houseflies (*Musca* sp.), 10 each of earwigs (*Euborellia annulipes*), roaches (*Pycnoscelus surinamensis*) and sow-bugs (*P. laevis*), all collected from a poultry yard where previous surveys had shown about 64 per cent of cysticercoid infection in amphipods, also revealed no parasites.

DEVELOPMENT OF LARVA IN THE FINAL HOST

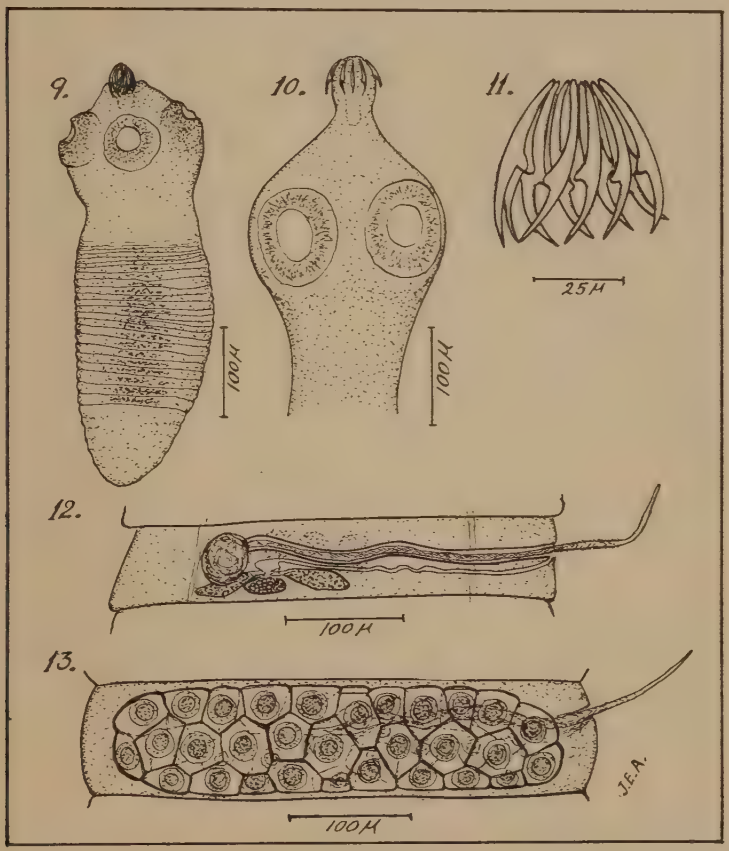
In experiments involving the development of the larvae in the final host, 100 or more completely formed cysticercoids were fed to each of a group of week-old laboratory-raised chicks, which were killed at various intervals thereafter. The results are shown in Table 1.

TABLE 1.—*Feeding experiments of week-old chicks with cysticercoids of* Hymenolepis exigua

No. of chick	Number of cysticercoids fed	Period of infection	Postmortem findings
1	about 100	6 hours	Many invaginated cysticercoids in gizzard and 1 in anterior portion of duodenum; the external membrane and tail of cysticercoids absent.
2	" "	10 "	1 invaginated cysticercoid in crop, 1 in proventriculus, and many in the gizzard; a few invaginated and evaginated cysticercoids in the duodenum.
3	" "	20 "	A few invaginated cysticercoids in the gizzard; a few invaginated and many evaginated cysticercoids in the duodenum.
4	" "	2 days	Many immature tapeworms (Fig. 9) about 0.5 to 1 mm long and 150 to 190 μ wide in the duodenum.
5	" "	7 "	Many immature tapeworms 2 to 3 mm long and 350 μ in maximum width in duodenum.
6	" "	10 "	Many mature and immature tapeworms 5 to 8 mm long and 410 to 470 μ wide in the duodenum, some containing gravid proglottids. (Eggs in droppings of bird 9 days after experimental infection.)
7	" "	15 "	As above. (Eggs in droppings of bird 10 days after experimental infection.)
8	" "	26 "	All mature tapeworms 8 to 10 mm long and 470 μ in maximum width in the duodenum. (Eggs in droppings of bird 9 days after experimental infection.)
9	293	45 "	216 mature tapeworms in the duodenum.
10	404	15 "	277 " " " " "
11	367	26 "	267 " " " " "

Chicks No. 6, 7, and 8 passed eggs in the droppings 9, 10, and 9 days, respectively, after experimental infection. This indicates the approximate time when the parasites reached maturity and the life cycle of the parasite was renewed.

Some observations were made relative to the number of cysticercoids that would establish and develop in the intestinal tract of the final host. Birds 9, 10, and 11, fed 293, 404, and 367 cysticercoids respectively, were found to harbor at autopsy 216 (73 per cent), 277 (69 per cent), and 267 (73 per cent) tapeworms. These results indicate that a large number of the cysticercoids ingested develop in birds.



Figs. 9–13. *Hymenolepis exigua.* Fig. 9. Young tapeworm, 2 days after infection. Fig. 10. Scolex of adult tapeworm. Fig. 11. Rostellar hooks. Fig. 12. Mature proglottid. Fig. 13. Gravid proglottid.

Although the longevity of these parasites has not been definitely worked out, tapeworm infection has been found to persist in a bird 6 months after experimental infection. The life span of tapeworms has been reported to vary from a few months to more than a year. Recently

Harwood (1938) reported that a chicken experimentally infected with *Raillietina cesticillus,* maintained the infection for at least 18½ months. On the other hand Jones (1931) reported a chicken infected with *R. cesticillus* lost its infection within 5 months. Wetzel (1934) also reported that a bird harboring *R. cesticillus* and passing segments, was negative on postmortem examination 121 days after experimental infection.

MORPHOLOGY OF THE ADULT

Hymenolepis exigua, Yoshida, 1908. Worms up to 16 mm long by approximately 470 μ wide. Scolex (Fig. 10) about 184 to 210 μ long and 206 to 225 μ wide. Suckers unarmed, about 70 to 104 μ in diameter; rostellum retractile, armed with a single row of 10 hooks (Fig. 11), each 42 to 45 μ long. Genital pores unilateral and slightly anterior to middle of segments. Segments broader than long (Figs. 12 and 13), gravid ones usually largest, about 95 μ long and 480 μ wide. Testes, 3 arranged in transverse row, obscured rapidly by developing ovary and uterus and at no time conspicuous in the strobila. Cirrus pouch long, extending about three-fourths breadth of segment, terminating in a large conspicuous seminal vesicle. Cirrus long, slender, often protruding from genital opening, armed with minute spines. Vagina ventral to cirrus pouch, opening into seminal receptacle, the latter between cirrus pouch and yolk gland. Ovary transversely elongate, somewhat bilobed. Uterus, sac-like, reticular, occupying practically entire segment, containing about 30 to 50 eggs. Eggs approximately spherical, 50 to 57 μ in diameter; onchosphere about 30 to 32 μ in diameter, possessing six hooklets, each about 13 μ long.

Individual gravid segments or groups of two or three were found at autopsy in the intestines of infected birds; the segments apparently disintegrated in the intestinal tract, allowing the reticular uterine sac containing the eggs to pass out with the droppings. The uterine sacs recovered from the droppings remained intact when kept in water, and it was in this form that they were placed on the soil in experiments involving the infection of the intermediate host.

SUMMARY

Experiments have been conducted on the development of the tapeworm *Hymenolepis exigua* in the intermediate host (amphipod, *Orchestia platensis*) and in the final host (chicken). Eggs of this parasite, when ingested by amphipods, were found to develop during a period of about 12 days to the cysticercoid stage capable of infecting chicks. Cysticercoids fed to week-old chicks developed to maturity in the duodenum and eggs passed in the droppings of the birds 9 to 10 days after experimental infection.

Sow-bugs (*Porcellio laevis*), beetles (*Tenebrioides nana*), houseflies (*Musca* sp.) and undetermined species of earthworms which were exposed to infection with eggs of *H. exigua* failed to become infected.

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A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS *POLYMORPHUS* AND NOTES ON THE STATUS OF THE NAME *PROFILICOLLIS**

HARLEY JONES VAN CLEAVE

In 1931, A. Meyer introduced the name *Profilicollis* into the acanthocephalan literature for a generic concept based on two species previously described as *Filicollis botulus* Van Cleave, 1916, and *F. arcticus* Van Cleave, 1920. Witenberg in 1932 (p. 260) regarded *Profilicollis* as a synonym of *Polymorphus*. Since 1931, the present writer has been engaged in a restudy of the species assigned to *Profilicollis* and of numerous undescribed forms from birds as well as the known species of *Polymorphus*.

When the name *Profilicollis* was first proposed, the distinctive features of its included species seemed so obvious that the writer felt impelled to accept the concept as valid, even after Witenberg expressed belief that *Profilicollis* is a direct synonym of *Polymorphus*. The relatively long, slender neck seemed clearly to differentiate the species from *Polymorphus*. Coupled with this was the observation that the concentric embryonic membranes of *Profilicollis* stood in contrast with embryos of *Polymorphus* which characteristically possess polar prolongations of the middle membrane. A reexamination of the original material on which *Filicollis arcticus* was based showed that among the embryos with strictly concentric membranes there were a few having polar prolongations. With the intergrading of these characters, this presumed generic distinction vanished, leaving length of neck as the only possible means of differentiating *Polymorphus* and *Profilicollis*. As a relative character this was found inadequate as a generic criterion. Numerous collections gave evidences supporting this decision, for intermediate forms were frequently encountered in this study. The only solution seemed to lie in the reduction of *Profilicollis* to a direct synonym of *Polymorphus*. Preliminary attention was directed to this synonymy in a brief abstract published in 1937 (Van Cleave, 1937: 563). By this decision *Profilicollis botulus* (Van Cleave, 1916) becomes *Polymorphus botulus* (Van Cleave, 1916) and *Profilicollis arcticus* (Van Cleave, 1920) becomes *Polymorphus arcticus* (Van Cleave, 1920).

In the second section of his monograph, Meyer (1933: 529) gave tacit acknowledgment of the inability to separate *Profilicollis* and *Polymorphus*, for when he prepared a key to the genera of Acanthocephala he

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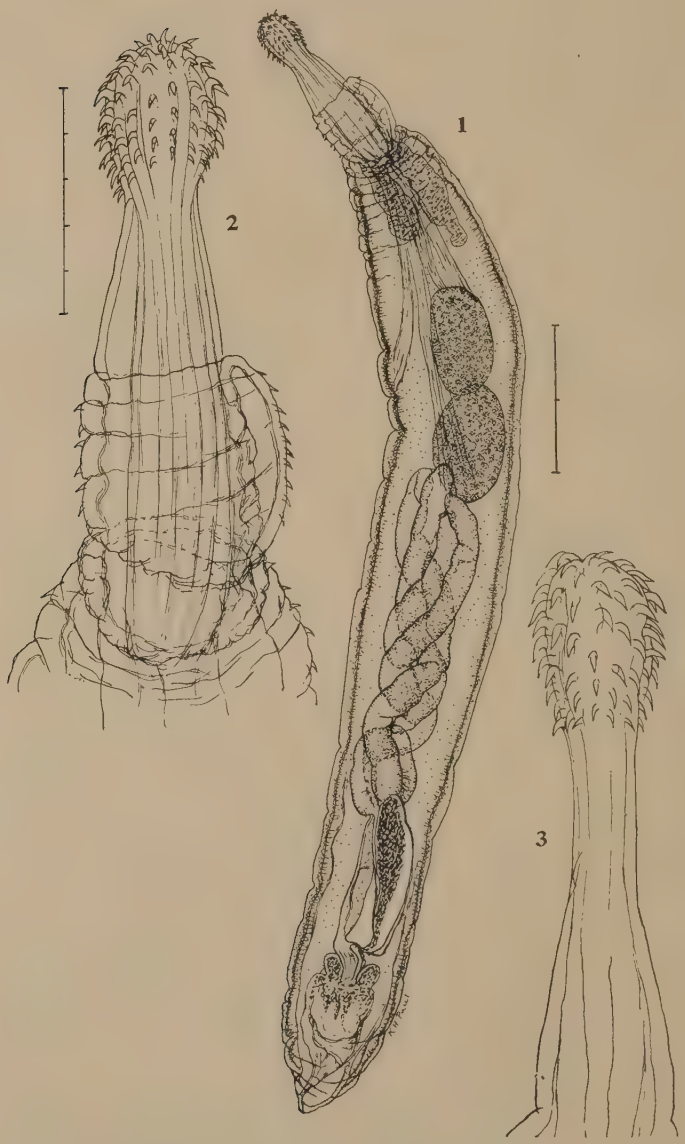

FIG. 1. Holotype male of *Polymorphus marilis*. The scale of magnification has the value of 1 mm.

FIG. 2. Proboscis, neck and spined anterior portion of body of holotype. Scale equals 0.5 mm.

FIG. 3. Anterior end of allotype female of *P. marilis*, drawn to the same scale as Fig. 2.

brought the two supposed genera down through the same alternatives as an unresolved complex bearing both the names without attempt at separation by key characters.

Among the specimens which led the writer to the foregoing decision on synonymy, were a group of seven worms (four males and three females) taken by Dr. Wm. P. Canavan from a scaup duck (*Nyroca marila*), near Oklahoma City, Okla. Though these were recognized as having many points in common with the species which had been ascribed to *Profilicollis,* they were at the same time clearly related to species in the genus *Polymorphus.* A new species recognized for these specimens is here described as *Polymorphus marilis.*

Polymorphus marilis n. sp.

(Figs. 1–3)

Body elongate, nearly cylindrical. Mature males 8 to 9 mm long with an average diameter of slightly less than 1 mm. Females 13 to 14 mm long with an average diameter of 1.5 mm. Neck long, slender, naked, retractable; when fully extended it may attain a length of 1.2 mm with a diameter of 0.35 mm at its base. Body spines small, confined to front end of body. Proboscis elongate ovoid, approximately 0.38 mm long by 0.23 to 0.27 mm wide at widest part; armed with 16 or 17 longitudinal rows of 7 or 8 hooks each. Hooks at base of proboscis 42 to 44 μ long; at tip 54 to 60 μ; on remainder of proboscis 57 to 66 μ. Proboscis receptacle 1.33 to 1.42 mm long. Lemnisci short, compact, about twice as long as receptacle. Male genitalia occupy about four-fifths the length of body cavity. Cement glands long and tubular. Embryos within gravid females 102 to 120 μ long by 12 to 17 μ in diameter, with polar prolongations of the middle membrane.

Type host: Nyroca marila (Linn.) taken at Oklahoma City, Okla., in April, 1935.

Holotype male (slide 2905.6), allotype female (slide 2905.3) and paratypes in collection of the writer at Urbana, Ill.

Polymorphus marilis differs from *P. corynosoma, P. botulus* and *P. arcticus* in size and shape of body, from *P. botulus* in size of the embryos and *P. arcticus* in the number of hooks on the proboscis. It differs from *P. minutus* in size and shape of body and length of neck; from *P. striatus, P. corynoides* and *P. mutabilis* in the number of hooks on the proboscis; from *P. contortus* and *P. crassus* in size of the hooks; from *P. magnus, P. phippsi,* and *P. obtusus* in size and proportions of the embryos.

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CERCARIA DONECERCA N. SP. (GORGODERID CERCARIA) FROM *MUSCULIUM PARTUMEIUM* (SAY), 1822

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In the examination of 37 clams, *Musculium partumeium,* two have been found infected with a new species of gorgoderid cercaria for which the name *Cercaria donecerca*[2] n. sp. is proposed.

The clams were collected from a small pond near Cold Spring on Hudson, N. Y., in June, 1938. The infected clams have each produced about fifteen to twenty-five cercariae per day for one month. Studies have been made upon both living and fixed specimens, the former being more satisfactory for morphological observations. Identification of the genital primordium was possible in material treated with an aqueous solution of methyl green and acetic acid, following the application of neutral red, a technique more or less specific for reproductive anlagen.

Cercaria donecerca n. sp.

(Figs. 1–7)

Total length of cercaria (Fig. 7) 1.3–1.6 mm; anterior chamber containing the young distome 0.19–0.26 mm by 0.16–0.19 mm; tail proper, 0.9–1.4 mm long, anterior portion of tail as wide as the chamber; tail gradually diminishes in width posteriorly, ending bluntly.

Body fusiform (Fig. 3), capable of great distension; emerges readily from chamber under slight pressure. Fully contracted body is 0.19–0.27 mm long by 0.10–0.14 mm wide; extended, 0.32–0.52 mm long by 0.03–0.09 mm wide. Body wall bears fine, parallel, longitudinal striations and fine transverse grooves. Sensory papillae distributed as follows: six around margin of acetabulum; four rows along ventral side, six papillae each in two median rows, eight papillae each in two lateral rows; arrangement of tubercles on oral sucker shown in Fig. 3.

On rim of acetabular opening peculiar "wart-like" sensory nodules (Fig. 3) 1–2 μ in diameter [in same position as sensory bristles reported from other gorgoderid cercariae by Sinitsin (1905) and Krull (1935)]; concentrated in region of sensory papillae, but extending also in scattered manner to acetabular edge.

Stylet, fragile, movable; measures 17–20 μ by 3–4 μ by 5–6 μ. Morphology shown in Figs. 4, 5, and 6. Oral suckers, 51–53 μ in diameter, vesiculated wall about 20 μ thick; sensory bristles and sensory nodules lacking. Acetabulum 62–65 μ in diameter; wall about 17 μ thick. Acetabular cavity about 30 μ in diameter; opening slit-like, at right angles to long axis of body in non-flattened condition, but may be circular under pressure.

Twelve unicellular penetration glands (Fig. 3), six on each side, located at level of acetabulum. Gland cells about 10 μ wide, nuclei about 3 μ in diameter. Granular

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[1] The writer wishes to thank Professor R. P. Hall for criticisms of the manuscript, and Dr. Paul Bartsch of the United States National Museum for identification of the molluscan host.

[2] Greek: from δονέω, shake, and Κέρκος, tail.

secretory material acidic, taking deep red stain with neutral red. Ducts inconspicuous in unstained worm; with neutral red, stained secretion enters ducts and renders them visible; secretion pours out as two long, deeply stained cords through separate pores on either side of stylet.

Oral aperture subterminal, 17 μ wide, usually crescentic, but may be circular. Oral cavity about 20 μ in diameter. Esophagus approximately 7 μ wide; midway between oral and ventral suckers it bifurcates to form digestive ceca which are 5 μ in diameter and extend to posterior tip of body.

Excretory pore terminal; bladder extends almost to acetabulum, where it divides to form two main collecting ducts; ducts extend to anterior border of acetabulum, where they divide; one branch turns back, and follows a tortuous course posteriorly. Flame cell pattern, 2[(4)+(4+4+4)]. Fifteen to twenty-five large cystogenous cells surround excretory bladder; secretory material composed of huge granules, 2 to 4 μ in diameter; granules stain brilliant crimson with neutral red.

Genital primordium (Fig. 3) immediately posterior to acetabulum. Consists of a well-defined, median, fusiform cell mass, about 30 μ by 10 μ in size, at right angles to long axis of body; another cell group, with ill-defined lateral projections, lies behind, but attached to the first. A third cell cluster lies at region of future genital pore; extending back from it is a string of cells, the future uterus, which disappears at level of middle of acetabulum.

The tail of the cercaria (Fig. 1), as in other gorgoderid cercariae, is the largest and most conspicuous part of the larva. The conical anterior chamber containing the young worm, is from one-sixth to one-eighth the length of the tail proper. The cercarial cavity in the chamber is ovoid, with a narrow opening apically. The anterior end of the worm is recurved to the posterior attachment point (Fig. 2). From the anterior chamber posteriorly, the tail gradually diminishes in size. The tail is distended with fluid, *i.e.*, its shape is not imparted by cellular elements. There is a small group of cells (Fig. 2), however, which attaches the distome to the tail. A thin cuticula covers the entire cercaria. Beneath the cuticula is a layer of very fine, parallel circular muscle fibers below which lie two lateral groups of larger, anastomosing, longitudinal muscle fibers. Arranged peripherally, are "mesenchyme-like" cells which are attached to the longitudinal muscle fibers.

The motion of the cercaria is peculiar. By alternate contraction of the longitudinal muscle fibers, the body is thrown into loose spirals. The reversal of the spiralling in newly emerged cercariae is about 100 to 120 per minute. The cercaria may float free in the water, anterior end down, or may attach itself to the substratum by the posterior tip.

Cercaria doncocerca is a member of the gorgoderid cercariae group. In another paper, Goodchild (1939) has reviewed the biology, and given a complete list of this group of cercariae. *C. doncocerca* differs from *C. gorgoderina vitelliloba*, *C. gorgodera cygnoides*, *C. gorgodera pagenstecheri*, *C. gorgodera varsoviensis*, *C. sphaerocerca*, *C. mitocerca*, and *C. conica* in the absence of an anterior enlargement of the tail; it differs from *C. gorgodera amplicava* in size, distribution of sensory tubercles, and absence of sensory bristles.

SUMMARY

A new species of gorgoderid cercaria, *C. donecerca,* has been found in the clam, *Musculium partumeium*. It possesses a large movable tail, divided into two regions: a conical, anterior chamber containing the young distome; and a terminal, cylindrical, tapering portion. The excretory system is described.

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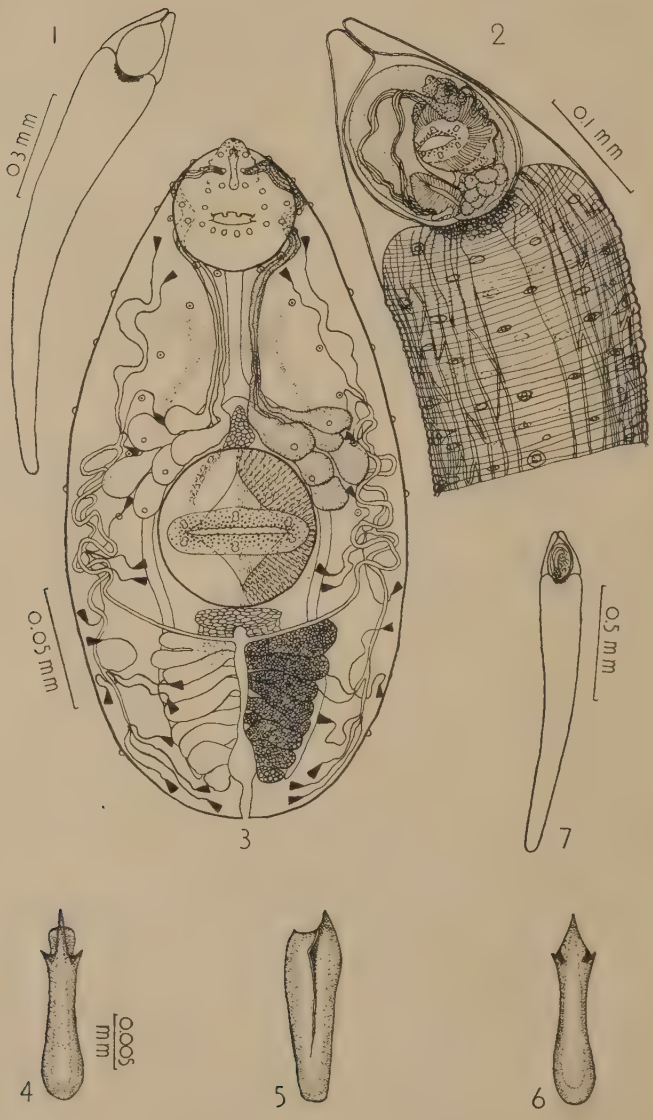
EXPLANATION OF PLATE, P. 136

FIG. 1. Cercarial tail.
FIG. 2. Anterior end of cercaria enlarged.
FIG. 3. Ventral aspect of distome, right side showing excretory system; left side detail of glands, etc.
FIG. 4. Stylet, ventral view.
FIG. 5. Stylet, lateral view.
FIG. 6. Stylet, dorsal view.
FIG. 7. Complete cercaria.



Cercaria donecerca n. sp.

FURTHER OBSERVATIONS ON ACQUIRED IMMUNITY TO THE TICK *DERMACENTOR VARIABILIS* SAY

WILLIAM TRAGER

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It has been shown (Trager, 1939) that guinea pigs and rabbits once infested with larvae of the tick *Dermacentor variabilis* acquire an effective immunity against these larvae. This immunity can be produced artificially by the inoculation of an extract of larval ticks and can be passively transferred. It depends to a large extent on the presence of a circulating antibody which accelerates a local cellular reaction which in turn walls off the tick larva from its normal food supply. Some additional information has now been obtained concerning two of the points left open in the previous paper.

I. *Immunization with various tick extracts.* Guinea pigs were inoculated intracutaneously on the left side with various tick tissue extracts, or with 0.85 per cent salt solution alone. By means of the pill box method previously described, larval ticks from a single batch of eggs were then applied to both sides and to the left ear of all the guinea pigs. Table 1 shows the results of such an experiment. It is clear that antigenic substance is present in the digestive tract and the cephalic glands of partly engorged ticks, as well as in the salivary glands of both fed and unfed ticks and in whole larval ticks. The cephalic gland extract, however, is not nearly as potent as the salivary gland extracts. It is worthy of note that during the course of the inoculations guinea pigs 4 and 6 showed marked reddening and hardening of the skin at the site of injection; guinea pigs 5, 9 and 10 showed a similar but milder reaction; while the other animals showed no local reaction whatever. Nevertheless, guinea pig 2 showed more immunity than guinea pig 4.

II. *The complement fixation reaction.* In the previous paper it was noted that guinea pig serum could not be used to demonstrate specific complement fixation because normal guinea pig serum reacted with tick extract antigen to fix complement. This difficulty has been overcome by using rabbit serum. Table 2 shows a typical result.

The complement fixation technic recommended by Taliaferro (1929) was followed. The reagents used were:

Antigen: Approximately 0.2 gm of larval ticks ground up in 2 cc of 0.85 per cent saline solution. Mixture centrifuged and supernatant diluted with saline to 25 cc.

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TABLE 1.—*Immunization of guinea pigs with various tick extracts*

	Guinea pig	Injected intracutaneously on left side: Amount	On Sept. 10, 11, 12 with Extract*	On Sept. 13, 14 with Extract*	Number of larvae engorged out of 100 applied on Sept. 22, 1938, to each of following regions: Left side	Right side	Left ear	Total number of engorged larvae
		cc						
Litter mates	1–♀	0.1	CG–1	CG–2	24	22	29	75
	2–♀	0.1	SG–1	SG–2	9	9	5	23
	3–♂	0.1	S	S	41	54	71	166
	4–♂	0.05	L	L	1	33	29	63
Litter mates	5–♀	0.1	SG–3	SG–4	3	13	9	25
	6–♀	0.1	L	L	0	13	11	24
	7–♀	0.1	S	S	46	47	22	115
	8–♂	0.1	SG–1	SG–2	36	25	36	97
Litter mates	9–♂	0.1	DT	DT	29	29	..	58
	10–♂	0.1	SG–3	SG–4	30	32	..	62
	11–♂	0.1	S	S	25	66	..	91

* The extracts were prepared by grinding various tissues in salt solution, as shown below. All the suspensions were centrifuged and the slightly turbid supernatant liquids used.

Extract	Tissue	Stage of tick	No. of ticks used	Vol. of 0.85% NaCl solution	Date of preparation
				cc	*September*
CG–1	Cephalic glands	Half-engorged adult ♀	4	1	9
CG–2	" "	" "	6	1	12
SG–1	Salivary glands	Unfed adult ♀	4	2	9
SG–2	" "	" "	12	1	12
L	Entire body	Larva	2000 approx.	6	9
SG–3	Salivary glands	Half-engorged adult ♀	4	2	9
SG–4	" "	" "	5	1	12
DT	Digestive tract	" "	4	2	9
S	0.85 per cent saline alone				

Amboceptor: Rabbit serum hemolytic for sheep cells in presence of complement.

Complement: Mixed serum from 3 normal guinea pigs.

Sheep red cells: These were washed 3 times with saline.

Test serums: Obtained from rabbits and inactivated by heating for one-half hour at 55° C.

The amboceptor was titrated first. On the basis of this titration a mixture of sheep cells, amboceptor and saline (so-called sensitized cells) was prepared such that the addition of 0.1 cc of it to 1.9 cc of other constituents would introduce two units of amboceptor and would give a red cell concentration of 0.5 per cent. In the presence of these concentrations of amboceptor and cells, the complement was then titrated. In the actual complement fixation tests, two units of complement, in this case 0.2 cc of a 1 : 10 dilution, 0.7 cc of antigen and 1 cc of the appropriate dilution of test serum were mixed and incubated one hour at 37° C. (In such a mixture the guinea pig serum used for complement was present

TABLE 2.—*Complement fixation in the presence of tick extract antigen and serum from rabbits previously infested with ticks*

Rabbit No.	Previous treatment	Serum dilution	Degree of complement fixation
1–♂	77 larvae engorged on rabbit June 7–14, 1938. Bled on Sept. 6, 1938.	1 : 4	+++
		1 : 8	++
		1 : 16	+
		1 : 32	±
		1 : 64	−
2–♂	44 nymphs engorged on rabbit Sept. 6–15, 1938. Bled on Sept. 22, 1938.	1 : 4	++++
		1 : 8	+++
		1 : 16	++
		1 : 32	±
		1 : 64	−
3–♂ Litter mate of 2	84 nymphs engorged on rabbit Sept. 6–15, 1938. Bled on Sept. 22, 1938.	1 : 4	++++
		1 : 8	++++
		1 : 16	+++
		1 : 32	++
		1 : 64	−
4–♂ Litter mate of 2	No ticks. Bled on Sept. 22, 1938.	1 : 4	±
		1 : 8	±
		1 : 16	±
		1 : 32	−
		1 : 64	−

at a dilution of 1 : 100, whereas 1 : 32 was the highest dilution of normal guinea pig serum which gave more than a trace of the non-specific complement fixation in the presence of tick extract.) Then 0.1 cc of sensitized cells was added to each tube, and the tubes were again incubated one hour at 37° C. Suitable controls showed that neither the antigen alone nor the test serums, at the concentrations used, had any anticomplementary action.

As shown in table 2, normal rabbit serum combined with tick extract did give a slight complement fixation (± indicates a trace of unhemolyzed red cells present), but this was negligible as compared with the high degree of complement fixation exhibited by the serums from previously infested rabbits, two of which were litter mates of the uninfested control.

SUMMARY

Guinea pigs can be partially immunized to larvae of *Dermacentor variabilis* by injecting them with extracts of the cephalic glands, salivary glands, or digestive tract of partially engorged adult female ticks, or of the salivary glands of unfed adult females.

The serum of rabbits previously infested with *D. variabilis* shows specific complement fixation with a larval tick extract antigen.

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DESCRIPTION OF *HAPTOPHRYA VIRGINIENSIS*, A PROTOZOAN PARASITE OF THE PICKEREL FROG, *RANA PALUSTRIS* LE CONTE

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Haptophrya virginiensis is a recently described species (Meyer, 1938) of astomatous ciliate found in the intestinal tract of the pickerel frog, *Rana palustris* Le Conte, a new host for the genus.

The intestinal tract of an infected frog was removed shortly after capture and placed in 0.7 per cent NaCl. Numerous specimens of *Haptophrya* were found in the rectum and posterior part of the small intestine.

Living organisms were examined with both compound and binocular dissecting microscopes. One specimen was kept under continuous observation for a 7-hour period. Camera lucida drawings and micrometer measurements were made from the living material.

The organisms remained in good condition for study for a period of approximately 24 hours. After that, plasmolysis occurred, the "neck" described by Woodhead (1928) developed, the animals became sluggish and soon died. Perhaps they would have remained in good condition for a longer time had a more dilute saline solution been used, as suggested by Woodhead (1928) and Bush (1934).

Permanent slides were prepared by fixing the material in Schaudinn's fluid and staining with Delafield's hematoxylin.

Haptophrya virginiensis has an oblong body, three to four times as long as broad, tapering slightly from the anterior end posteriorly. Five free-swimming individuals, selected as typical, had an average length of 354 μ and an average width of 95 μ at the anterior end and 73 μ at the posterior end. The organism is broadly rounded anteriorly and has a truncate to rounded posterior end. The body length is much less than the range of 1.260 to 1.600 mm given by Cépède (1910) for *Haptophrya gigantea* and the average length of 1.286 mm for *H. michiganensis* given

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by Woodhead (1928). It does, however, fall within the range of 200 to 1580 μ given by Bush (1934) for the latter species. No individual in the entire collection even approximated the body length given as characteristic for those species.

Many of the organisms were free-swimming or so soon lost their attachment to the intestinal wall as to appear so. Their movement was rather slow but steady and was accomplished by the beating of the fine cilia covering the body. No "worm-like undulations" described by Bush (1934) in *H. michiganensis* were observed in this species. Though the organisms appeared to be unable to change their form spontaneously, the pellicle, which is under sufficient tension to maintain a definite body contour, is flexible, bending when an object is touched but immediately returning to its normal position after the contact ceases. The presence of fine pellicular striations was called to the writer's attention by Dr. D. H. Wenrich. This fact should be noted in considering the species diagnosis previously published (Meyer, 1938).

The length of the body cilia is uniform, about 3.5 μ. This is slightly less than the 5 μ given by Bush (1934) for the cilia of *H. michiganensis* though approximately the same as the 3.8 μ recorded by Woodhead (1928) for the length of body cilia in the same species. The cilia on the margin of the ventrally located sucking disc are longer and heavier than those on the body proper. This appears to be true of all species.

The three most conspicuous structures in the body of *Haptophrya virginiensis,* and probably in the other species of the genus also, are the sucking disc, macronucleus, and contractile vacuole (Fig. 1).

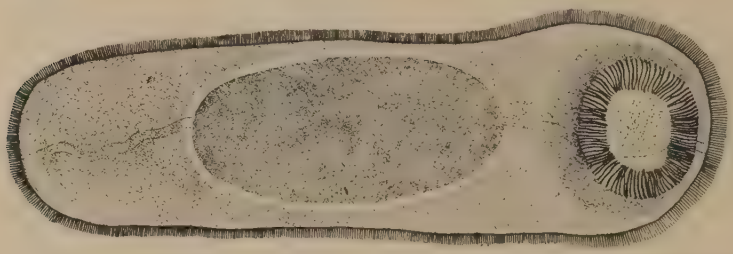

FIG. 1. Ventral view of *Haptophrya virginiensis* drawn from living specimen with aid of camera lucida. Note antero-ventral sucking disc, prominent macronucleus, and canalicular contractile vacuole. Magnification ×336.

The sucking disc is a cup-shaped depression on the antero-ventral surface of the cell. It serves as an organ of attachment. The rim of the disc is provided with strong cilia that are at least twice the length of those of the body proper. In proportion to the size of the entire animal, the disc in *H. virginiensis* seems to be larger than that in *H. michiganensis.*

The large macronucleus is elongate ovate to ellipsoidal and may be found in various positions within the endoplasm. It is easily visible in living cells. A ratio of approximately 3:1 for length of body to length of macronucleus appears to hold in both living and fixed material. Though the length of the macronucleus of *H. virginiensis* falls within the size range given by Woodhead (1928) and Bush (1934) for the corresponding structure in *H. michiganensis,* its width is relatively much greater.

It is probable that the micronuclei lie within the nuclear membrane as in *H. michiganensis* (Bush, 1934). They could not be observed in the living condition and were not distinctly differentiated in the fixed material.

The contractile vacuole consists of a canal which extends the length of the animal on the dorsal side and which appears to communicate with the exterior by clearly defined pores. It is a permanent structure that may be nearly straight or irregularly sinuous. The pores are arranged in a linear series and are spaced at definite intervals along the canal rather than at the anterior and posterior ends only as in *Haptophrya planariarum* (Bush, 1933).

Asexual reproduction is by catenular budding. The method as described by Maupas (1879) and Cépède (1910) for *Haptophrya gigantea* and by Woodhead (1928) for *H. michiganensis* is followed very closely by *H. virginiensis.* One organism divides into two and division is repeated by the segments so formed with a chain of individuals resulting. These later become detached. In *H. virginiensis* no more than six animals were observed in such a series. The observations in this connection closely resemble those of Woodhead (1928) for *H. michiganensis.* By far the greater number of organisms observed were single individuals; relatively few were in process of division.

The genus *Haptophrya* was established by Stein (1867) as one of four subdivisions of the somewhat heterogeneous group of organisms previously referred to the single genus *Opalina.* Four other species have been included in the genus. *Haptophrya planariarum* von Siebold (1845) is found in the triclad, *Planaria torva. H. gigantea* Maupas (1879) is found in *Bufo pantherinus, Discoglossus pictus,* and occasionally in *Rana esculenta. H. tritonis* Certes (1879) was found by Raphael Blanchard in *Tritonis alpestris* but the validity of this species was questioned by Bütschli (1889) on the grounds that the organism so described "möglicherweise ein Balantidium sein könnte." *H. michiganensis* Woodhead (1928) was discovered by F. N. Blanchard in *Hemidactylium scutatum* and in one specimen of *Amblystoma jeffersonianum.* This is the only species, in addition to *H. virginiensis,* yet described from American material.

SUMMARY

1. The morphology of *Haptophrya virginiensis*, an astomatous protozoan parasite from the intestinal tract of the pickerel frog, *Rana palustris* Le Conte, is described.

2. The body is shorter and the macronucleus is relatively larger and broader than in *H. michiganensis* Woodhead.

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THE LARVA OF *PSOROPHORA* (*JANTHINOSOMA*) *HORRIDA* DYAR & KNAB (DIPTERA: CULICIDAE)

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Psorophora horrida is listed as a synonym of *P. champerico* in Dyar's 1928 monograph of American mosquitoes, but Matheson (1934) examined specimens of *P. horrida* sent to him from Arkansas by H. H. Schwardt, and proved that this mosquito is a valid species. Matheson described the peculiar male terminalia, but apparently nothing is known concerning the immature stages. The purpose of this paper is to record a few observations regarding the breeding habits of *P. horrida* and to describe the larva.

The larvae were collected August 1, 1938, in Mohawk Park, Tulsa, Oklahoma, four days following a heavy rain. Part of Mohawk Park is composed of a level, wooded area, several acres in extent, and in this location there are a number of shallow depressions which hold water for several days following a rain. *P. horrida* larvae were breeding in two of these temporary rain pools. In 100 dips, 30 fourth stage larvae and one pupa were found, and with them were taken many pupae of *P. columbiae, P. signipennis, P. cyanescens, Aedes vexans, A. trivittatus,* and a few larvae and one pupa of *P. ferox.* Several second stage larvae of *Anopheles punctipennis* were also collected. As all but one of the *P. horrida* were in the larval stage, it appears that the mosquito develops a little more slowly than the other species collected, excepting *P. ferox.* No doubt its eggs were deposited in the depressions before the rain, as were those of the other *Psorophora* and *Aedes* collected.

P. horrida seems to require heavily shaded pools for breeding. In Mohawk Park the larvae were found only in shaded puddles, and not in nearby puddles exposed to the sun, although other species were present. I have collected large numbers of *P. ciliata, columbiae, discolor, signipennis,* and *cyanescens* in both shaded and unshaded puddles, but I have never found *P. horrida* or *ferox* in water exposed to full sunlight.

The larvae collected in Mohawk Park were taken to the laboratory, and several of them were bred out separately. The identification of females obtained from the larvae was confirmed by Dr. Alan Stone, of the U. S. National Museum, while the male terminalia agree in all respects with Matheson's description.

DESCRIPTION OF LARVA

Head broader than long, bulging laterally. Antennae curved, somewhat swollen basally and tapering distally, longer than the head; spinulate; a small multiple tuft

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beyond the middle; one short and three long spines, and a short papilla, at the tip. Anteantennal hairs of 5 to 7 branches; upper and lower head hairs double. Antennal, anteantennal, and head hairs with very fine, short, lateral branchlets.

Lateral abdominal hairs multiple; long on first two segments and shorter on succeeding segments. On the third segment these hairs have 3 to 7 branches, while on segments four, five, and six they have 3 to 5, rarely 2, branches.

Comb scales of eighth abdominal segment seven in number, arranged in an arc at posterior edge of a weakly chitinized plate. Each scale has a long central spine flanked on each side by one or two smaller ones. Posterior to the comb scales are three hairs, the outer two multiple and the middle one double or single.

FIG. 1. Head of larva.

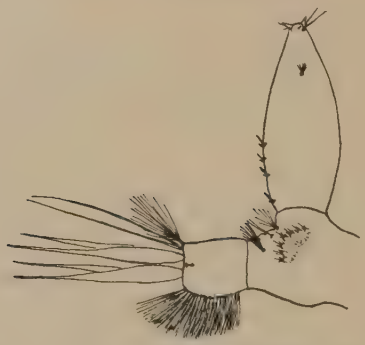

FIG. 2. Terminal abdominal segments of larva.

Air tube inflated, approximately 1×3, with three or four short pecten spines on basal third; a very small, multiple tuft laterally on apical third; and several short and two long hairs at apex.

Anal segment longer than wide, ringed by the plate, which is perforated ventrally by the hairs forming the ventral brush. Dorsal brush a long hair and a multiple tuft on each side. Lateral hair very small, usually split apically into several branches, but occasionally it may be single. Anal gills long and tapering.

The larva of *P. horrida* resembles that of *P. ferox* very closely, but may be distinguished from the latter by the lateral abdominal hairs of the third to sixth segments. Grabham (1906), in his description of *Janthin-*

osoma echinata, states, "Lateral hairs of the abdomen paired and flattened; on the anterior segments each hair is large and 4- to 7-branched, hairs becoming smaller and with fewer branches on the hinder segments." Howard, Dyar, and Knab (1917) include Grabham's description in their discussion of *P. posticatus*. However, these authors state that these hairs on the larva of *P. sayi* are "multiple on first segment, double on second, single on third to sixth." *J. echinata*, *P. posticatus*, and *P. sayi* are listed by Dyar (1928) as being synonyms of *P. ferox*. The differences in the number of branches of the lateral abdominal hairs may be due to individual variations, but *P. ferox* larvae collected in Oklahoma, as well as those I collected in Panama, all possess multiple lateral hairs on the first and second abdominal segments, while on the third segment these hairs have 2 to 4 branches, and are long and single, very rarely double, on the fourth, fifth, and sixth segments.

The base of the antenna of *P. horrida* is slightly more swollen than in the case of *P. ferox*, but this character is not a satisfactory one for separating the two species.

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THE ENZYME UREASE AND THE OCCURRENCE OF AMMONIA IN MAGGOT-INFECTED WOUNDS*

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When an open wound becomes infected with blowfly maggots it commonly develops an odor of ammonia, and the same thing has been observed when maggots feed upon exposed meat or animal debris. The production of ammonia under such conditions has been a matter of physiological interest for many years.

The subject appears, in addition, to be of some economic importance. According to Laake (1938) the female flies of the screwworm, *Cochliomyia americana,* lay their eggs more abundantly in wounds which are alkaline than in those with an acid reaction. This, of course, has a harmful effect in increasing the screwworm infection in a wound.

On the other hand, in the medical use of the blowfly maggots *Lucilia sericata* and *Phormia regina* it was found by Baer (1931), and later confirmed by others, that wounds with an alkaline reaction healed faster than those inclined to be acid. Messer and McClellan (1935), in their study of the hydrogen-ion concentration of wounds treated with maggots noted: "In all cases the fluctuations in pH corresponded roughly with the clinical condition of the wound; the progress of healthy healing being accompanied by a consistent increase in alkalinity, while failure to progress toward healing . . . was followed by a marked fluctuation toward acidity."

ON THE ORIGIN OF AMMONIA IN MAGGOT EXCRETIONS

Weinland (1905) observed that a considerable amount of ammonia occurred in the excretions of blowfly maggots and thought it came from the deamination of amino acids present. Hobson (1931) also noted a vigorous production of ammonia when maggots fed upon meat, but found no evidence of ammonia through deamination. He added, "The origin of ammonia remains an open question." Brown and Farber (1936), in a later work, found that maggots do produce some ammonia by this process. Messer and McClellan (1935) stated that the source of ammonia was not definitely known, but they were inclined to believe that the alkalinity arose through the deamination of amino groups set free by the maggots.

In their natural surroundings blowfly maggots feed commonly upon decaying animal material, and their alimentary tract is of course highly

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contaminated with bacteria of various kinds. Van Tieghem (1864) showed that many species of bacteria can produce ammonia. Therefore, with normal maggots, that is, those which live under nonsterile conditions, there is always the question as to what part bacterial infection plays in the formation of ammonia. Brown (1936) believed, however, that the bacterial ammonia is inconsiderable compared with that produced by the maggots.

Whether or not the ammonia is produced entirely by the maggots themselves or also by the bacteria present in the alimentary tract, the fact has been established that the excretions of maggots do contain a large amount of ammonia. Also when this ammonia, together with the ammonia-producing enzymes, is excreted into the wounds in which the maggots feed, it is sufficient to account for the alkalinity observed.

THE ENZYME UREASE CAUSES PRODUCTION OF AMMONIA

Another source of ammonia, and a prolific one, is the breakdown of urea by the enzyme urease. This enzyme attacks only urea, with the prompt and abundant production of ammonium carbonate. There is no lack of urea for this enzyme to act upon, as urea is of course commonly present in all animal tissues. The enzyme urease has also been found in many kinds of organisms, including bacteria, fungi, green plants, and in animals, both vertebrate and invertebrate.

Urease is not one of the more recently discovered enzymes, as it has been known to science for over sixty years. An immense amount of study has been devoted to the chemistry of urease, but references to this enzyme appear to be rare in entomological literature.

Owing to the numerous reports of vigorous production of ammonia by maggots, together with the occurrence of both urea and urease in living tissues, it was thought by the writers that urease might occur in maggots and be responsible for the evolution of some of the ammonia in their excretions.

UREASE OCCURS IN MAGGOT TISSUES

A search was accordingly made for the presence of this enzyme in maggots. The species used in the tests was the blowfly *Lucilia sericata*, the one which a few years ago had been largely used medically in the treatment of non-healing wounds. The well-known method of ammonia determination described by Marshall (1913a, b) and modified by Van Slyke and Cullen (1914b; 1916) was used. This is a standard test for the presence of urease. Both the necessary apparatus and the technique used are described in detail by the latter authors.

The Van Slyke and Cullen method was developed by them largely for the determination of urea in urine. Their technique required the addition of the enzyme urease to the sample of urine. Its action in splitting

the urea produced an amount of ammonia which was determined quantitatively, thus showing the concentration of urea. In the present work a reversal of their technique was employed, by the addition of urea to determine the presence of the enzyme. This is a common and accepted method.

The material used in the present series was obtained in the following manner: Normal or nonsterile maggots were allowed to grow to maturity and migrate to clean sand. They were thus much cleaner than if taken directly from the food. For each test ten larvae were used, their combined weight being about 500 milligrams. They were washed, dried, and macerated in one-half gram of chemically clean quartz sand. The resulting viscous mass was placed in the standard incubation tubes. The number of tubes were divided into two parts, one for the test series and the other to serve as checks. One hundred milligrams of urea were placed in each of the test tubes, to be used as the substrate for the urease; the checks, of course, received none. The amount of urea to be added is more or less arbitrary, as Van Slyke and Cullen (1914a) have shown by mathematical calculations that, within wide margins, the amount of ammonia produced by urease is not affected by the amount of urea present but only by the concentration of the enzyme itself.

In each of the tubes 5 drops of toluene were used as the bacteriostat. The incubation solution was 10 cc of M/10 potassium phosphate buffers that were adjusted to approximately pH 7.4. The tubes were incubated for 24 hours at a temperature of 35° to 36° C. The standard tests as described by Van Slyke and Cullen for ammonia were then made.

The activity of urea in macerated non-sterile maggots is shown in Table 1. It can be seen that a vigorous production of ammonia was brought about in all cases where urea was added. The explanation of

TABLE 1.—*Presence of urease in macerated non-sterile maggots*

Experiment no.	Weight of maggots used	Urea added	No urea added
		N/50 HCl neutralized	N/50 HCl neutralized
	mg	*cc*	*cc*
1–T	485	73.5	
1–C	482		5.6
2–T	469	62.1	
2–C	512		4.3
3–T	510	79.7	
3–C	496		5.8
4–T	502	76.4	
4–C	491		4.9
5–T	495	70.4	
5–C	506		5.7
6–T	476	73.5	
6–C	481		4.7

this effect is, of course, that the enzyme urease, which can attack no other substance than urea (Armstrong, Benjamin and Horton 1913; Takeuchi 1909), hydrolyzes the extra urea which is added, thus producing am-

monia and thereby showing its presence. The tests were repeated with similar results many more times than shown in the table.

Determinations were also made of the urease content of the natural excretions of living non-sterile 2nd- and 3rd-instar maggots. A large number of active maggots were removed from their food, washed and placed in a Buchner funnel and allowed to drain for several hours into a beaker. During this period, to facilitate drainage, the maggots were occasionally sprayed lightly with distilled water from an atomizer.

The excretions were divided into two series of tests as before, incubated, and tested in the same way. The results (Table 2) show that the

TABLE 2.—*Urease in excretions of non-sterile maggots*

Experiment no.	Weight of excretions used	Urea added	No urea added
		N/50 HCl neutralized	N/50 HCl neutralized
	mg	*cc*	*cc*
1–T	945	12.3	
1–C	961		1.2
2–T	960	14.5	
2–C	942		1.5
3–T	948	16.2	
3–C	955		2.1
4–T	980	14.1	
4–C	964		1.9
5–T	953	15.7	
5–C	987		1.6

enzyme urease is present also in the excretions. It appears to be less in quantity than in the macerated tissues. In reality this is not so because the excretions were considerably diluted with water as part of the process of securing them. The enzyme is thus excreted into the wound, and undoubtedly some of the ammonia in the wound is formed there by the action of urease on urea in the tissues of the wound.

The experiments were then extended to include determinations of the urease content of maggots reared under the usual sterile conditions. The method used in the production of the maggots was that described by Simmons (1934) and involved the use of aseptic technique throughout the process. It included the sterilization of the eggs, placing them upon sterile food, and rearing the maggots in the absence of contamination. Checks upon sterility of the maggots were made in suitable aerobic and anaerobic cultures. Maceration of the maggots and the procurement of the excretions were done in a manner similar to the non-sterile tests except with aseptic precautions. At this stage checks for sterility were again made.

Maggots produced aseptically as described were also found to contain urease (Table 3). It was considerably less abundant than in non-sterile maggots, which is to be expected in the absence of bacteria usually contaminating the alimentary tract.

The presence of ammonia in the excretions of maggots reared under sterile conditions has been indicated by other workers. Hobson (1932)

TABLE 3.—*Urease in maggots reared under aseptic technique*

Experiment no.	Condition of material	Weight of material	Urea added	No urea added
			N/50 HCl neutralized	N/50 HCl neutralized
		mg	cc	cc
1–T	Macerated tissue	485	22.1	
1–C	"	492		1.8
2–T	"	496	18.7	
2–C	"	510		1.2
3–T	Excretions	792	10.8	
3–C	"	984		0.7
4–T	"	965	9.9	
4–C	"	978		0.5

noted the development of ammonia by sterile maggots and adds that the amount is distinctly less than in non-sterile maggots. Michelbacher, Hoskins, and Herms (1932) noted the occurrence of ammonia in the excretions of maggots which they had reared aseptically. Messer and McClellan (1935) determined quantitatively the ammonia produced by maggots grown under sterile conditions and concluded that the amount given off by the usual application of maggots in a wound is sufficient to account for the alkalinity of the wounds under observation.

SOME ADDITIONAL FACTS ABOUT THE ENZYME UREASE

Pasteur (1861) made one of the earliest discoveries in connection with this enzyme. He observed that ammonia was produced in the fermentation of urine and that this was caused by a living organism. Shortly afterwards Van Tieghem (1864), one of his students, isolated the organism and named it *Micrococcus ureae*. Later Van Tieghem found that the breakdown of urea with the formation of ammonia was brought about by an enzyme which the bacteria produced, and the enzyme was called "urease."

Since that time several species of pus-forming bacteria have been found to elaborate this enzyme, and certain types of fungi also produce it. Many kinds of green plants, widely differing in their classification and habits, contain urease in various parts of their structure but largely in their seeds. In this respect the legumes are outstanding and the seeds of the soybean and jackbean are now the principal sources of commercial urease. Recently Damodaran and Sivaramakrishnan (1937) have found the seeds of the common watermelon to be rich in urease.

In animals urease occurs throughout a wide range as in plants, having been found present in them from the lowest of invertebrates up to and including man, in whom it occurs in various organs of the body.

There is a certain amount of historical interest in the fact that urease was the first enzyme ever to be isolated in pure crystalline form. It had been known, of course, for many years, but only by its activity in breaking down urea. Sumner (1926) succeeded in isolating the enzyme from

the mass of other protein materials with which it is always associated, and he obtained it in pure crystalline form. The publication of his discovery led to a storm of controversy and doubt, but later the accuracy of his findings became well established. He found it to be protein in nature and a much more complex molecule than that of urea, upon which it acts. Sumner, Gralen and Eriksson-Quensel (1938) have recently determined that urease is an exceedingly large and heavy molecule, having a molecular weight of 483,000. Urea with a weight of 60 is in striking contrast.

In the determination of how enzymes, in general, function, crystalline urease has been used to a great extent, as it is free from impurities. A number of valuable observations have been made, such for instance as the work of Van Slyke and Cullen (1914a), in which they found that urease attacks urea first by uniting with a molecule of urea and secondly by disrupting the molecule. Immediately after breaking down one molecule of urea the enzyme attacks another without any measurable loss of time, and so on. One molecule of urease can therefore disrupt many molecules of urea, and speed of urea destruction is not dependent upon the amount of urea present but upon the concentration of the enzyme.

Urease is a very sensitive enzyme and the speed with which it reacts is easily disturbed. A number of substances will stop its activity altogether and many others will increase its velocity. Some of these materials occur in living tissues.

The end-products of the destruction of urea by urease are carbon dioxide and ammonia, and strangely enough both these substances profoundly affect its activity. Still more remarkable is the fact that these end-products influence urease in opposite directions; carbon dioxide considerably accelerates its speed and ammonia greatly retards it. This, according to Armstrong, Benjamin and Horton (1913), is "unprecedented" in the chemistry of enzymes.

SUMMARY

The larvae of *Lucilia sericata*, recently used as surgical maggots, contain the enzyme urease in their tissues and excretions. Urease is present both in non-sterile maggots and in those reared under aseptic conditions. This enzyme, in breaking down urea, produces ammonia abundantly in maggot-infected wounds.

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ADDENDUM

Since this article was sent to press, another has appeared (Brown, 1938, Biochem. J. **32**: 903) in which that author states he was unable to find urease in this insect. Instead of using fresh, normal tissue for his analysis, Brown used "acetone powder" preparations. One of us (F. C. B.) has found that such preparations contain little if any active urease.

BLOOD ALTERATIONS IN TYPHLOHEPATITIS OF TURKEYS, WITH NOTES ON THE DISEASE

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INTRODUCTION

While working with a blood protozoon of turkeys which was reported by Johnson *et al.* (1938), we became interested in certain round forms in stained smears of turkey blood containing this protozoon, which were taken from birds that at autopsy were found to be affected with typhlohepatitis or so-called blackhead disease. This observation was not unusual during the autumn months, but, since blackhead disease is most common among growing turkeys at that time of year, this finding was looked upon as being coincidental rather than as having related significance in the cause of the disease. An effort to determine any such possible relationship was prompted by discovering a sudden marked increase of such rounded forms in blood smears taken in April from a female turkey that had carried comparatively few of the blood protozoa during the winter months, and which at this stage developed blackhead. Fig. 1 is a photomicrograph of a blood smear containing these rounded forms. The size and general shape of these are quite similar to certain stages of *Histomonas meleagridis* found in livers of turkeys affected with blackhead. It was decided, therefore, to study the blood of naturally and artificially infected cases of blackhead disease to determine if these rounded forms might be certain stages of *H. meleagridis*. A study of the blood in blackhead disease, likewise appears of importance since this phase seems to have been neglected in previous studies.

REVIEW OF LITERATURE

With the exception of the report by Smith (1895), in which he mentions the occurrence of flagellates in the blood of turkeys affected with blackhead, we have found no mention in the literature of a possible relationship between blood parasites and *H. meleagridis*. According to the drawings by Smith, the parasite spoken of as a flagellate appears similar, if not identical with the blood parasite described by Johnson *et al.* (1938). In discussing the blood of turkeys affected with blackhead, Smith reports that the blood was examined "of many of the cases . . . before the bird was killed in order to detect any existing blood infection. No disease of the blood was observed, however, excepting in No. 16, in

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which a pronounced leucocytosis was present." From a study of the lesions it was concluded that, "The primary seat of the disease is in the ceca. From these the liver is invaded. Other organs are not attacked. The affection of the cecum is due primarily to the multiplication of the micro-organisms within the walls of the cecum . . . chiefly in the mucous membrane or sub-mucous tissue." The opinion was expressed that, "the micro-parasites are conveyed by the blood directly from the diseased ceca into the liver and there deposited in different places where they begin to multiply and spread in all directions, thereby forming the spherical foci of disease which appear as circles on the surface of the liver."

The pathology of blackhead disease of turkeys has been quite completely reported by numerous workers whose findings are essentially in agreement with those of Tyzzer (1919, 1934). The assumption that the causative organisms are conveyed by the blood from the primary lesions in the ceca to the liver seems to have been carried over from the hypothetical explanation by Smith, but so far it does not appear that anyone has studied the blood extensively to see if this actually takes place. Our findings in a study of the blood of naturally and artificially infected cases of blackhead are herein reported and illustrated.

MATERIALS AND METHODS

Twenty-one poults were hatched in an incubator in our laboratory on May 1, 1937. These were brooded in a thoroughly disinfected small fly-proof brooder house until May 29, 1937, when they were placed in a specially constructed cage in our animal house. This cage was 4×7 feet, having a raised 1×2 inch mesh 12 gauge electrically welded wire floor, and the entire cage covered with cheesecloth of 24×28 strands to the inch construction. The cloth was protected from within by ordinary chicken wire to prevent injury by the turkeys, and from without by wire screening 16 meshes to the inch, to prevent injury from any other source. This type of cheesecloth had previously been found in our work to be an effective barrier against *Simulium nigroparvum* (Twinn), the insect vector found capable of transmitting the blood protozoon, which in blood smears appears similar to *Leucocytozoon smithi*.

Blood smears were made at six weeks of age and then at intervals of about three weeks to check the condition of the birds. Blood smears were made daily, usually for five days before infective material was administered to determine whether the preliminary blood picture was normal and uniform, and to secure an average, by which subsequent blood smears could be compared.

In attempting to infect the experimental turkeys with blackhead the following means were used: subcutaneous implantation of diseased liver tissue; feeding of diseased tissue; oral and rectal administration of his-

tomonal cultures, and similar administration of cecal discharges from "carrier chickens," in which no protozoa other than *H. meleagridis* were found.

Subcutaneous inoculations were accomplished by inserting about 2 cc of diseased liver tissue beneath the skin in a pocket formed by blunt dissection between the skin and the breast muscle. Material administered *per os* was placed directly into the crop by means of a glass tube and a glass rod stilette. Approximately 20–30 grams of extensively diseased portions of liver were coarsely chopped and inserted into the crop in this manner. Infective material was introduced into the rectum by means of a small short rubber tube to which a funnel was attached. In some cases turkeys thus treated were partially suspended by the feet, but with some support against the breast, for approximately an hour. Histomonal cultures and cecal discharges of "carrier chickens" were introduced either *per os* or per rectum or by both of these avenues.

Following inoculation with infective materials the birds were placed in another similarly constructed cage, and blood smears made daily, with few exceptions, until death or destruction.

In administration of the infective materials every precaution was taken to keep the inoculum warm and fresh to insure viability.

Blood smears were stained with Wright's stain. All percentages listed for differential white cell counts are on the basis of a count of at least 500 cells. In some cases they are based on a count of 1000 cells. Tissues were fixed in Helly's fluid and stained with dilute Delafield's hematoxylin and Azure II plus eosin.

EXPERIMENTAL RESULTS

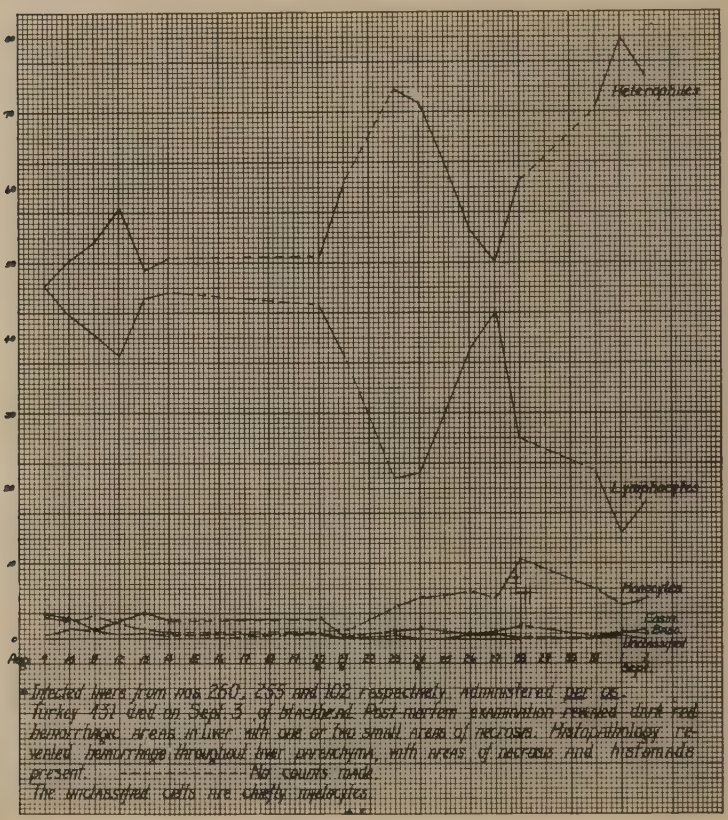
Table 1 gives the average of 65 differential leucocyte counts of 14 turkeys prior to inoculation with infective materials, also the variations

TABLE 1.—*Differential leucocyte counts from 14 experimental turkeys preparatory to inoculation with blackhead materials*

Bird No.	No. of counts	Hetero-philes	Eosino-philes	Basophiles	Lympho-cytes	Monocytes
401	2	35.5	1.4	1.6	60.1	1.4
408	6	50.3	1.0	2.3	42.0	4.4
416	1	41.0	0.4	4.0	52.8	1.8
422	5	40.3	0.9	5.0	52.1	1.7
423	5	32.6	0.7	3.0	61.8	1.9
426	5	37.1	2.0	3.0	55.9	2.0
429	5	39.0	1.5	4.0	54.0	1.5
431	7	51.1	0.8	2.7	43.5	1.9
444	4	53.5	0.3	3.5	41.5	1.2
452	5	45.1	0.5	3.7	48.5	2.2
454	5	54.8	0.6	2.2	41.3	1.1
455	5	42.9	0.9	3.4	50.8	2.0
459	5	44.8	1.1	4.8	47.5	1.8
460	5	40.0	0.6	1.0	56.5	1.9
Variation in individual counts		30.0 – 58.0	0 – 2.2	0.8 – 7.0	37.8 – 64.2	0.8 – 3.2
Average		43.4	0.9	3.2	50.6	1.9

encountered for the various cells. The cells listed as heterophiles are the polymorphonuclear eosinophilic granulocytes or granulocytes containing rod-shaped eosinophilic granules. From this table it may be noted that the average percentages of different leucocytes in healthy turkeys kept under the conditions of this experiment are as follows: Heterophiles 43.4, eosinophiles 0.9, basophiles 3.2, small and large lymphocytes 50.6, and monocytes 1.9, with the occurrence of occasional degenerated basophiles.

Graph 1 illustrates the results of daily leucocyte counts of turkey No.

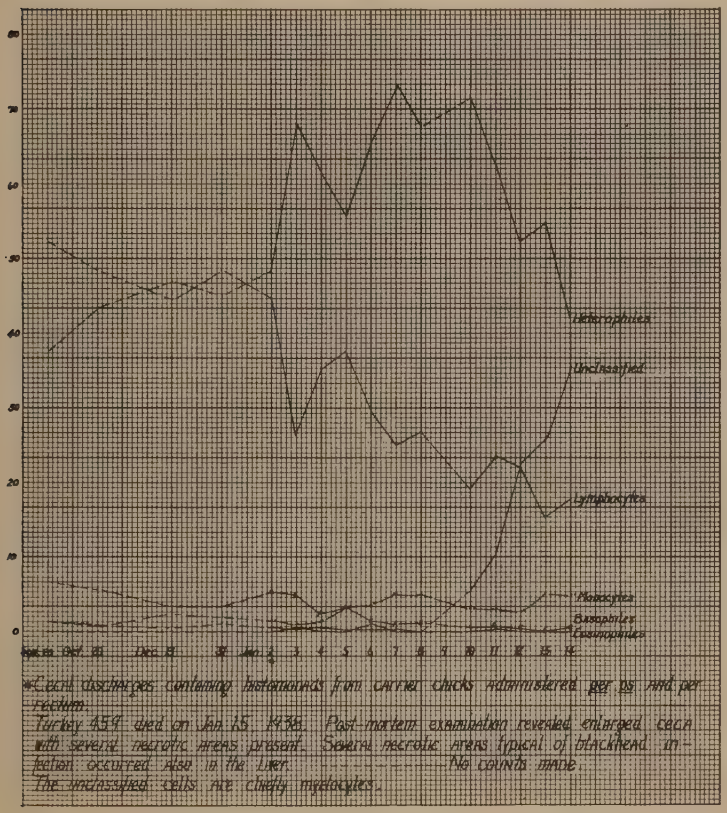

Heterophiles
Lymphocytes
Monocytes
Eosin.
Baso.
Unclassified
Aug.
Sept.
Infected meals from nos 260, 255 and 402 respectively administered per os.
Turkey 431 died on Sept. 3 of blackhead. Post mortem examination revealed diffuse red hemorrhagic areas in liver with one or two small areas of necrosis. Histopathology revealed hemorrhage throughout liver parenchyma, with areas of necrosis and histomonads present.
No counts made
The unclassified cells are chiefly mediocytes

GRAPH 1. Differential leucocyte counts of turkey 431.

431 before and after infection. The persistent increase in heterophiles noted in all affected birds is interrupted in this bird on three days, with a drop to what would be considered normal levels, as indicated by the valley in the curve. This drop undoubtedly indicates a transient re-

covery, as the heterophiles again increase in numbers as the disease apparently flares up and finally terminates fatally.

Graph 2 illustrates the data on turkey No. 459. Here it may be noted


Heterophiles
Unclassified
Lymphocytes
Monocytes
Eosinophiles
Basophiles
*Cecal discharges containing heterakoids from carrier chicks administered per os and per rectum.
Turkey 459 died on Jan 15. 1938. Post mortem examination revealed enlarged ceca with several necrotic areas present. Several necrotic areas typical of blackhead infection occurred also in the liver. No counts made.
The unclassified cells are chiefly myelocytes.

GRAPH 2. Differential leucocyte counts of turkey 459.

that the increased heterophiles continue from the time of infection until three days before death. Actually this increase is maintained until the fatal termination, as the unclassified cells are chiefly immature heterophiles or myelocytes and, therefore, on the last few days these immature cells may be included with the heterophiles.

Fig. 2 illustrates a blood smear from a typical case of artificially produced blackhead in which a marked increase in heterophiles may be noted.

Table 2 includes data on ten cases of naturally occurring blackhead

TABLE 2.—*Differential leucocyte counts of ten cases natural blackhead terminating fatally. Blood taken for counts in each case a few hours before death*

Blood cells	Bird numbers										Average
	101	*67*	*179*	*232*	*1GR*	*161*	*222*	*112*	*274*	*1BR*	
Heterophiles ...	62	71	74	79	79	80	82	89	89	91	80
Eosinophiles ...	0	0	0	0	0	0	0	0	0	0	0
Basophiles	0	1	1	1	1	0	0	0	2	0	1
Lymphocytes ...	25	14	22	18	17	12	13	5	8	7	14
Monocytes	6	2	3	1	3	2	3	2	1	1	2
* Unclassified ..	7	12	0	1	0	6	2	4	0	1	3

* Chiefly myelocytes or immature heterophiles.

which terminated fatally. In comparing the average leucocyte counts in this table with the counts in the graphs of experimentally infected birds immediately before the fatal termination, it may be noted that the percentages of the various cells are in close agreement.

A very interesting lesion occurred in the proventriculus of turkey No. 454 which is an experimental bird infected by feeding blackhead liver. We are not aware of a previous report of blackhead lesions in the proventriculus. Fig. 3 is a photograph of a drawing of a stained section from the lesion in this organ. It may be noted from this that an extensive area of necrosis extends from the mucosa, through the gland lobules, and into the muscular wall. Fig. 4 illustrates the histomonads preceding the necrosis by moving and multiplying rapidly between the muscle fibers.

This bird also had lesions in the liver but none in the ceca. It may be assumed, therefore, that the primary infection was in the proventriculus. It is conceivable that the histomonads were carried from this lesion to the liver and that support is here found to the hypothesis that in blackhead disease the histomonads are conveyed by the blood from the primary seat of infection in the ceca to the liver. However, a careful study of stained sections from all these birds has failed to reveal these organisms either within the blood vessels or in the peripheral blood. On the other hand countless numbers of these may be seen in and around the necrotic lesions produced. To assume that these were conveyed by the portal circulation from the proventriculus to the liver and there filtered

TABLE 3.—*Differential leucocyte counts of naturally occurring cases of blackhead confirmed by post-mortem examination. Blood for counts taken in each case immediately before death*

Blood cells	Blood protozoa absent						Blood protozoa present				
	Bird numbers						Bird numbers				
	231	*255*	*175*	*260*	*102*	*280*	*128*	*247*	*297*	*64*	*137*
Heterophiles	76	80	81	85	93	93	75	80	80	81	91
Eosinophiles	0	0	1	0	0	0	0	0	0	0	0
Basophiles	1	0	0	1	0	0	0	0	0	0	0
Lymphocytes	17	15	9	11	6	3	20	14	8	14	6
Monocytes	3	3	1	2	0	2	5	6	4	5	3
*Unclassified	3	2	8	1	1	2	0	0	8	0	0

* Chiefly myelocytes or immature heterophiles.

from the blood would also seem to entail considerable organ selectivity which does not maintain when implantations are made. By this method of administration lesions may be produced in the adjacent muscles and in the lungs.

As mentioned at the outset we were interested in finding out if there was any relationship between the blood protozoon, resembling so-called leucocytozoa, and *H. meleagridis*. Table 3 gives blood counts of naturally occurring cases of blackhead, some with blood protozoa and some without. No significant difference in blood counts was found in the two groups.

DISCUSSION

The administration of diseased blackhead tissue by various channels to this group of experimental turkeys was attended by the numerous failures which have characterized the results of other investigators. Thus, of the seven turkeys that received 12 feedings of diseased tissue by mouth only two became infected with the disease. Tyzzer and Collier (1925) succeeded in infecting seven of 18 turkeys as the results of injections of fresh infected liver tissue. These birds, however, varied in age from five to 15 days, while the turkeys in this experiment were from four to six months old at the time infected material was fed. It is known that susceptibility to blackhead decreases with age.

The importance of trauma of the cecal wall in reducing the resistance of the mucosa to blackhead infection has been referred to by various investigators. Injury to the cecal mucosa is effected by the common cecal worm, and the freedom from this parasite may account in part for the difficulty experienced in infecting laboratory turkeys. Moreover, in natural infection birds undoubtedly have opportunity to ingest the highly pathogenic form of the organisms when in the flagellate phase. When fed diseased tissue the animal must first liberate the tissue forms which subsequently must develop into the flagellate phase in order to penetrate the cecal mucosa.

Two out of four animals developed systemic blackhead as a result of subcutaneous implantation of diseased liver tissue. It is interesting to note that when this method is used the lungs become invaded by the parasites and lesions are set up in these organs.

Histomonads in cecal evacuations of "carrier chicks" proved to be much more pathogenic than those in other material even though all the turkeys receiving this type of material were almost eight months of age. One feeding of this material resulted in infection in one instance. On the other hand, a single dose of cecal evacuations, from "carrier chicks," administered per rectum while in a suspended position, resulted in infection in three cases. One was destroyed and two died. The disease in the animals that succumbed to this treatment was characterized in each

instance by markedly uniform lesions in both ceca as well as in the liver. The incubation period was of short duration since bird number 408 was found to have marked lesions four days after administration of this material.

The high degree of pathogenicity noted in the birds that were treated while in a suspended position may be explained on the assumption that while the bird is in this position the parasites have the optimum conditions for actually establishing themselves directly in cecal mucosa in a short time after administration of the material. The hypothesis that the cecal mucosa is invaded soon after the infective material is administered is borne out by an examination of the marked changes that occur in the differential leucocyte counts within 24 hours following this treatment. Note bird 459 in graph 2. The cells noted as unclassified are chiefly myelocytes.

Differential leucocyte counts of blood from three turkeys that developed blackhead as the result of infective material by mouth revealed a marked heterophilia, and a severe but irregular monocytosis. In two that were fed infected liver tissue a rise in heterophiles of 8 and 9 per cent took place in the first 24 hours. In turkey number 429 that was fed cecal discharges from "carrier chicks" just once, a rise of 21 per cent in heterophiles occurred within 24 hours. This marked increase in heterophiles within 24 hours after feeding the infective material was noted in all four birds that received cecal evacuations from "carrier chicks." Only one of these, turkey number 459, had any degree of monocytosis. The heterophilia, however, became so severe that it completely masked the percentages of other cells. The three birds in this group whose blood was studied daily until death developed a marked myelocytosis which started approximately five days before death. The percentages of myelocytes appearing in the 24 hours immediately preceding death were 23, 13, and 35 per cent respectively.

This sudden heterophilia appearing within 24 hours after infection allows considerable interesting speculation concerning this infection. From a study of sections of ceca it appears certain that the histomonads penetrate the cecal epithelium and become established in a parenteral position within 24 hours. This injury to the mucosa hardly appears sufficient to explain the generalized heterophilia since there is no marked local mobilization of these cells. The possibility exists that as the parasites multiply and produce pressure necrosis of the host tissue there is liberation of toxic substances which stimulate the formation of heterophiles.

In the later stages of the disease there may be marked destruction of both heterophiles and erythrocytes resulting in the appearance of mye-

locytes and erythroblasts, as well as other immature stages of these series of cells.

In this investigation a large number of blood smears was carefully examined. Determinations were based on counts of at least 500 cells from each smear. In no case were any intravascular forms of protozoa found.

SUMMARY

Histomonas meleagridis in cecal discharges of "carrier chicks," when introduced into the rectum of turkeys held in a suspended position, is extremely pathogenic as compared to this protozoon in other material and to other channels of administration.

The average differential leucocyte count of normal turkeys maintained under the conditions of this experiment is as follows: heterophiles 43.4, eosinophiles 0.9, basophiles 3.2, lymphocytes 50.6, and monocytes 1.9 per cent.

Experimental blackhead disease of turkeys results in a marked disturbance in the blood picture which is characterized by: 1. A marked heterophilia of from eight to 21 per cent increase within 24 hours of receiving the infective material. 2. A persistence of the heterophilia until death. 3. An irregular monocytosis in some cases, especially in those receiving subcutaneous implantations of infective material. 4. Myelocytosis and anemia usually preceding the fatal termination. The sudden marked heterophilia following introduction of infective material indicates that the histomonads penetrate the cecal epithelium and establish themselves in some parenteral position where toxic material results within 24 hours.

Natural cases of typhlohepatitis were found to have very similar leucocyte disturbances to those found in the experimentally infected birds.

An extensive lesion in the proventriculus due to the administration of *H. meleagridis* by way of the mouth, is described and illustrated.

In no case could intravascular protozoan forms be demonstrated in the experimentally infected birds. The rounded forms illustrated in Fig. 1 are interpreted as macrogamete stages of a blood protozoon resembling *L. smithi* and are not related to *H. meleagridis*.

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Explanation of Plate

Photomicrographs with initial magnification ×750.

Fig. 1. Rounded forms of a blood protozoon in peripheral blood of turkey affected with typhlohepatitis. This work indicates that these are not related to *H. meleagridis*.

Fig. 2. Blood smear of bird affected with blackhead disease. Note large number of heterophiles in one oil-immersion field.

Fig. 3. A drawing of a cross section of a portion of the proventriculus of turkey number 454. The lumen is indicated at A, while b,b,b marks an extensive area of necrosis obliterating the glandular mucosa and gland lobules, extending through the muscular wall beside the square marked "a" and into the center of an acute area to the serosa at c.

Fig. 4. Oil-immersion field taken at square marked *a* in Fig. 3 to show forms of *H. meleagridis* in large numbers between muscle fibers, and preceding the necrosis.

1
2
3
4
A
b
b
b
c

OBSERVATIONS ON *HENNEGUYA SALMINICOLA* WARD, A MYXOSPORIDIAN PARASITIC IN PACIFIC SALMON*

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I. GENERAL CONSIDERATIONS

Henneguya salminicola was described in 1919 by Dr. H. B. Ward from cysts found in the body musculature of a silver salmon (*Oncorhynchus kisutch* Walbaum) taken from the Stickeen River in southeastern Alaska. Ward described the cysts as "pyriform, fairly uniform in size, and hard to the touch. . . . The cysts measured 3 to 6 mm in diameter and were found everywhere through the muscle mass."

Zschokke and Heitz (1914) had previously encountered cysts measuring 3 to 5 mm in a silver salmon during a parasitological survey of salmonid fishes of the Kamchatka peninsula. Myxosporidian spores within the cysts were identified by these workers as those of *Henneguya zschokkei* Gurley, although they gave no details as to the spores found and it seems quite probable that Kudo (1920) was correct in his assumption that these spores belonged to the same species which was later described by Ward as *H. salminicola.*

During the past four years, various species of Pacific salmon have been received by the U. S. Bureau of Fisheries Pathology Laboratory at Seattle with requests for information concerning the white cysts, colloquially known as "tapioca" because of their superficial resemblance to a tapioca pearl, to be found therein. A microscopical examination of these cysts has invariably revealed the spores of *Henneguya salminicola.* The sources of infected material have ranged, geographically, from the Columbia River to southeastern Alaska. However, the geographical distribution of a parasite based upon the site of recovery is quite meaningless when applied to as migratory a host as the Pacific salmon, which may well acquire an infection passing through an endemic area located many hundreds of miles from the point of subsequent capture. The species of salmon in which this parasite has been found are the pink salmon (*Oncorhynchus gorbuscha* Walbaum), the silver salmon (*O. kisutch* Walbaum) and the chinook salmon (*O. tschawytscha* Walbaum).

A somewhat detailed study of fresh material taken from a silver salmon caught in Puget Sound and preserved material collected from various pink salmon of southeastern Alaska confirmed Ward's findings and also contributed several additional facts concerning this species.

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The cysts are small, white in color, and encapsulated by connective tissue laid down by the host. They measure from 3 to 7 mm in diameter. They vary in firmness according to the thickness of their connective tissue capsule which, in turn, is probably dependent upon the duration of the infection. The cysts appear to be scattered throughout the body muscles with a noticeable concentration of cysts in the area below the dorsal fin. In all cases observed, the cysts were deeply embedded in the body musculature and were filled with a white viscous fluid which contained only mature spores. The cysts are found in the layers of fascia binding the muscles and when removed, leave a definite impression in the muscle tissue.

The spores are quite typical of the genus *Henneguya,* being composed of two symmetrical valves enclosing two polar capsules and the sporoplasm. Each valve is prolonged posteriorly to form a long process or tail. A sutural ridge encircles the spores in a straight line although it may be thrown into a series of very small folds. Ward described the spores of this species as possessing six or seven folds along the sutural ridge. When these folds are present, they number from five to seven although an examination of living spores revealed 70 per cent possessing the folds and in the remaining 30 per cent, the sutural ridge was perfectly smooth. There are no striations on the shell valves.

The spores of *H. salminicola* contain a large iodinophilous vacuole which is easily seen in stained preparations or in living and formalin-preserved spores treated with weak iodine solution. This vacuole is faintly visible in the living unstained spore as a spherical area slightly lighter in color than the surrounding cytoplasm.

The two polar capsules, each with an independent foramen, are separated by an isthmus diverging posteriorly. As noted by Ward, one polar capsule is significantly longer than the other although the breadths of both capsules average the same. The polar filaments contained within the polar capsules are but faintly discernible in formalin-preserved spores. When stained with iron hematoxylin or Giemsa, the wall of the polar capsule stains deeply and totally obscures the filaments. The filaments apparently make five or six large loops within the polar capsule.

The sporoplasm occupies most of the spore cavity and extends well up between the polar capsules. The sporoplasm contains two nuclei although additional nuclei may be seen within the spore and are apparently valve or capsulogenous nuclei.

The tails of the spores were, in all instances, unequal in length, the difference between the longer and shorter tail averaging 6.2 microns.

II. BIOMETRIC DATA ON *H. salminicola*

To determine the biometric limits for this species, dimensions were recorded by means of a camera lucida on a sample of 200 living spores

from the silver salmon; on 200 spores from the same source preserved for three months in ten per cent formalin; on 200 spores from pink salmon preserved for 18 months in an unknown mixture of formalin and alcohol; and on 200 spores taken from the silver salmon, fixed in Schaudinn's fluid, and stained in the usual manner with Heidenhain's iron hematoxylin and eosin. The arithmetic means and standard errors of the taxonomically important dimensions, as determined from this series of measurements, are listed in Table 1.

TABLE 1.—*Biometric data on* Henneguya salminicola; *all measurements in microns*

	Living spores	Stained spores
Length of longer polar capsule	4.57 ± .04	4.35 ± .03
Length of shorter polar capsule	4.19 ± .04	3.71 ± .03
Breadth of longer polar capsule	2.73 ± .03	2.29 ± .02
Breadth of shorter polar capsule	2.70 ± .04	2.29 ± .02
Length of longer tail	34.35 ± .27	32.71 ± .29
Length of shorter tail	28.14 ± .25	25.51 ± .21
Length of spore cavity	10.37 ± .09	9.27 ± .05
"X" length	13.51 ± .08	11.96 ± .06
Breadth of spore	8.94 ± .05	7.27 ± .03

The results show the customary shrinkage noted by Kudo (1921) in stained and mounted spores. However, no significant difference was observed between the living spores and those preserved in ten per cent formalin or the formalin-alcohol mixture.

In general, these data show slightly smaller dimensions for *H. salminicola* than were recorded by Ward. It is possible that this difference is due to the small number of individuals measured by Ward as the range of these data, in all instances, overlapped those given by him.

III. DISCUSSION

Unfortunately, little reliable information is available regarding such factors as the life history, incidence of infection, and host-parasite relations of *H. salminicola.* All available data concerning these factors have been obtained from various fisheries' investigators who have encountered infections of *H. salminicola* during the course of their work or from cannerymen who have been sufficiently curious about the cysts found in their fish to send material to this laboratory. However, observations from all sources tend to agree in certain particulars and as the data have quantitative, rather than qualitative, limitations, some tentative conclusions seem justifiable.

In addition to the three species of salmon already mentioned, the chum salmon (*O. keta* Walbaum) is also reported subject to natural infection. Of the five species of Pacific salmon, apparently the sockeye (*O. nerka* Walbaum) alone enjoys freedom from this parasite. This condition may well result from the food habits of the sockeye which, unlike other salmon, feed primarily upon plankton throughout their

entire life. The pink salmon reputedly is most seriously affected by *H. salminicola,* one competent observer placing the incidence of infection as high as ten per cent of all the fish comprising the later runs into certain limited areas of southeastern Alaska. It is quite certain that only an occasional silver, chinook, or chum salmon carries the infection.

On the basis of available evidence, southeastern Alaska is the endemic area of infection for this parasite. Whether the Pacific salmon are natural, or accidental, hosts from *H. salminicola* is not known. Certainly, the paucity of infections reported from the chum, chinook, and silver salmon would indicate these species to be accidental hosts. Whether or not this is likewise true for the pink salmon, with its significantly higher incidence of infection, is a debatable point which can only be settled by additional facts.

IV. SUMMARY

1. Cysts of *Henneguya salminicola* Ward have been found in the body musculature of the silver salmon (*Oncorhynchus kisutch* Walbaum), the pink salmon (*O. gorbuscha* Walbaum), and the chinook salmon (*O. tschawytscha* Walbaum). They have likewise been reported, although not confirmed by microscopical examination, from the chum salmon (*O. keta* Walbaum). Cysts of this parasite form the so-called "tapioca" found in the body musculature of the salmon.

2. Spores of *Henneguya salminicola* are typical of the two-tailed species of this genus. As found by Ward, they are sufficiently different from all other known species to warrant specific ranking.

3. Biometric data for the taxonomically important dimensions of the spores of *Henneguya salminicola* are recorded.

4. From the evidence now available, the endemic area of infection in Pacific salmon by *Henneguya salminicola* is centered in southeastern Alaska.

5. The chum, silver, and chinook salmon are believed to be accidental hosts for *Henneguya salminicola.* Whether or not the pink salmon represents the true host cannot be determined from data now available.

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STUDIES ON OXYURIASIS. XX. THE DISTRIBUTION OF THE OVA OF *ENTEROBIUS VERMICULARIS* IN HOUSEHOLD DUST

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As part of a study of the incidence of *Enterobius vermicularis* in persons residing in or near Washington, D. C. (1, 2), an investigation was made of the distribution of pinworm ova in various households. In the majority of cases, pinworm infection is a familial condition, as pointed out by Wright and Cram (3), a fact not at all surprising when one considers the ease with which infective material may be scattered in a household. The extent of the scattering of the ova has been only surmised, as no detailed study of the actual distribution of ova in a home has been made previously. Our method consisted of the collection of dust samples from various parts of the houses studied and the subsequent examination of the samples in the laboratory.

The only other examination of dust for pinworm ova known to us is that made by Oleinikov (4). He collected dust from the floors, benches and window sills of a schoolroom, with a brush (apparently a camel's-hair brush), dipped in a dilute solution of glycerin. The brush was washed into a test tube and the washings centrifuged. One to several pinworm ova were found in a microscopic field. In several schools, Oleinikov also used the brush on bed linen and on the inside of children's underwear and recovered many ova.

DESCRIPTION OF HOUSEHOLDS

Seven households were examined by us. Two important considerations entered into their selection, namely, at least one member of the household must have a heavy infection with pinworms, and the mother of the family must be cooperative. Six of the seven houses were occupied by white families, one by a negro family. Three families were of a high and the remaining four were of a medium-low social-economic level. All of the families had a somewhat similar history in that one or several of the children were known to be infected with pinworms before the mother established contact with us either directly or through the pinworm clinic maintained by the Division of Zoology at Providence Hospital, Washington, D. C.

Before the dust examination of a household was undertaken, an attempt was made to determine, by the use of the NIH swab, how many

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members were infected with pinworms. In two instances, the father of the family refused to use the swab. The results of the swab examinations are given in Table 1.

TABLE 1.—*Results of swab examinations of members of households in which dust was collected*

Household	Adults			Children			Total		
	Number swabbed	Number not swabbed	Positive	Number swabbed	Number not swabbed	Positive	Number of members	Positive	Number of heavy infections
I	1	1	1	4	0	4	6	5	4
II	2	0	1	3	0	3	5	4	1
III	2	0	1	2	0	2	4	3	2
IV	3	0	2	2	0	1	5	3	1
V	2	1	0	6	0	4	9	4	1
VI	2	0	2	3	0	3	5	5	5
VII	3	0	3	5	0	4	8	7	1

Five of the homes, in which dust was collected, were in Washington, D. C., one in Alexandria, Virginia, and one in Arlington, Virginia. All but one were houses consisting of living room, dining room, kitchen, two to four bedrooms, and bathroom. The exception was an apartment of three rooms and bath, consisting of a combination living room and bedroom, one bedroom, and a kitchen which was also used as a dining room. Five of the homes were clean and well kept, as judged by ordinary standards. In the remaining two there was obviously an accumulation of dust and dirt of long standing. All of the houses had been occupied by the present families for at least a year; the apartment had been occupied less than four months. Although it was impossible to find out with any degree of accuracy the date of onset of the pinworm infection in all of the homes, it was ascertained that all the infections were of at least several months' standing.

EXPERIMENTAL METHODS

Our dust samples were collected with a small camel's-hair brush, which had been inserted into a perforated rubber stopper fitted into a test tube. The brush was moistened with water, passed over the surface from which a sample was to be taken, then placed in a test tube and returned to the laboratory for examination. Several different techniques, both for sedimenting and floating the ova, were tried and abandoned as being inefficient and time consuming, for it was found that no sedimentation or flotation technique would entirely free pinworm ova from either the fluffy textile fibres or the heavier inorganic material composing the dust sample. A very simple technique was finally adopted. The brush was dipped gently into a few drops of decinormal sodium hydroxide solution on a deep-well slide and this was examined under the compound microscope. If ova were not found on the first examination, the same

procedure was repeated; in most of the positive cases, at least one ovum was found on the initial examination. When it was desired to test the viability of the ova by hatching, artificial digestive juice was used instead of N/10 NaOH. After examination, the slide containing ova was placed in a moist chamber to prevent evaporation, and incubated at 37° C. After a period of one to several hours the slide was removed from the incubator and reexamined for hatched larvae. Ova of *Enterobius vermicularis* are readily distinguished from those of the oxyurids that infect such household pests as rats, mice and cockroaches. We found none of these oxyurid ova in our dust samples. In one instance, we found a whipworm egg collected from the middle molding of a bedroom door.

RESULTS OF EXAMINATIONS

We collected and examined a total of 241 dust samples. In the households studied, samples were collected from all the rooms, including the kitchen and bathroom. Collections were made from locations and objects at various levels where dust usually settles, including the floor, baseboard, table legs and tops, chairs, davenport, standing lamps, dresser and cabinet tops, shelves, picture frames, window sills and frames, top molding of doorways and windows, and ceiling lights and chandeliers when these were present. In the bedrooms, samples were also collected from the sheets, mattress and framework of the beds, and in the bathroom from the tub, wash basin, and toilet seat. In several households, samples were collected from the children's toys and outer play garments.

Pinworm ova were found in 221, or 91.7 per cent, of the 241 dust samples examined. The ova were recovered from all the rooms at all levels. The 20 negative samples appear to be of little significance since other samples from the same room were positive and since samples from corresponding locations in other rooms were positive. We made no attempt to determine the total number of ova present in any dust sample. When a sample was found positive on the first examination, no further examination was made, so that any quantitative comparisons would be misleading. But, as was to be expected, more ova per single examination were found in the bedrooms than in any of the other rooms.

The ova recovered from the dust samples were in all stages of development, from tadpole to fully developed embryos. About one-quarter of the ova proved to be so deteriorated that their stage of development was uncertain; of the determinable ova, more than three-quarters contained fully developed embryos and only about one-sixth were still in the tadpole stage. Although only 15 of the ova contained embryos that were motile when examination was made, we considered, on the basis of their appearance under the microscope, that about half of the ova were either still viable, though inactive, or had died within a short time prior to the

examination. Our data show no correlation between distribution in any part of the house and the condition of the ova or their stage of development.

In order to check on our microscopic determinations of the condition of the ova, 24 were selected at random from those which had been classified as probably viable, though not motile. These were incubated in digestive juice at 37° C with the following results:

Not hatched	9
Not hatched but active	1
Partially or completely hatched	14

One of the latter group proved particularly interesting. It was obtained from a dust sample collected from the top molding of a doorway and placed in the incubator at 11:20 A M on Oct. 12. On examination at 1:45 P M on the same day, the ovum had hatched and the active larva was transferred from the digestive juice to Ringer's solution. It was kept in this medium in the icebox during the night and in the incubator during the day and remained motile under these conditions until the late afternoon of October 14.

DISCUSSION

The most significant feature of these studies appears to be the discovery that *Enterobius vermicularis* ova are present in dust at all levels in all rooms of homes housing one or more heavily infected individuals. The occurrence of these ova at lower levels, such as on the floor, chairs, beds or other pieces of furniture, might be conceivably the result of contamination directly from the clothing or persons of infected individuals, but the common occurrence of ova at higher levels, such as the top molding of doorways, ceiling lights, etc., points definitely to the transportation of the ova by air currents. Although many of the ova from these higher levels were dead and deteriorated, it seems to us improbable that transportation in the air is largely confined to more or less desiccated ova of relatively low specific gravity, since several viable ova were found at higher levels in locations where direct human contamination seems to be eliminated. Viable ova, therefore, on occasion can be carried in the air; indeed the occurrence of dead ova in the samples from the higher levels may reflect simply the long intervals between cleanings of these more inaccessible places and the consequent death of ova which were viable when deposited.

The conclusion that viable ova may be present in the air throughout a household tends to support the beliefs of Oleinikov (4) and Lentze (5) that infection with pinworms can be acquired by inhalation of dust containing embryonated ova. Oleinikov's evidence was largely negative, since it consisted chiefly in the persistence of pinworm infection in certain adults in spite of great personal cleanliness. He reported also that pin-

worm ova were present in the nasal secretion of some children and that stubborn infections were common in children who were mouth breathers or who swallowed their nasal secretion. Wilhelmi and Quast (6) also found ova in the nasal secretion of children but believed that the ova reached the nasal passage through manual contamination. Lentze, however, experimentally proved that viable pinworm ova could remain suspended in the air for periods up to two minutes, and that such eggs could be recovered from the nasal mucus after the experiment.

Granting that our survey is by no means exhaustive, it nevertheless seems to indicate that viable ova are carried in the air of infected households, and that the possibility of reinfection by inhalation of the ova must be considered in prophylaxis and in the therapy of individuals in such households.

SUMMARY

Ova of *Enterobius vermicularis* were found in dust collected at all levels in all of the rooms of 7 houses occupied by one or more persons heavily infected with pinworms. The ova, in part at least, must have been carried by air currents. Infection by inhalation is therefore theoretically possible and should be considered in the prophylaxis and therapy of oxyuriasis.

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RESEARCH NOTES

PRELIMINARY NOTE ON THE LIFE HISTORY OF *SUBULURA BRUMPTI*, A COMMON CECAL NEMATODE OF POULTRY IN HAWAII

Studies on cecal worms parasitizing chickens in Hawaii have shown that *Subulura brumpti* is of common occurrence, having been found in 42 out of 50 sets of ceca examined from birds obtained in Honolulu markets. As far as is known to the writer, the life history of no member of the genus *Subulura* has been reported heretofore.

Recent examinations of the body cavities of various insects collected in poultry farms have shown some of them, reported below, to be commonly infected with encysted nematode larvae (Fig. 1) having bulbous esophagi and conspicuous

Fig. 1. Encysted infective larva of *Subulura brumpti* recovered from the body cavity of the beetle *Alphitobius diaperinus*.

esophageal valves resembling those noted in the adult subulurids. Three of these encysted larvae measured as follows: length, 450 to 465 μ; maximum width, 126 to 135 μ; distance of excretory pore and nerve ring from the anterior end, 70 to 74 μ and 65 to 70 μ respectively; pharynx, 18 μ long; esophagus, 180 to 200 μ long; tail, 44 to 50 μ long.

A large number of these encysted larvae were fed to four laboratory-raised chicks; another four chicks of the same group of birds were kept under the same environmental conditions as controls. Two of the experimentally infected chicks, killed 6 days after infection, harbored in the ceca a large number of developing subulurid larvae, 2.3 to 2.4 mm long by 126 μ wide. The other two infected chicks, killed 3 months after experimental infection, were found to harbor adult specimens of *Subulura brumpti*. The four control birds were also killed and were found negative for all helminths.

In order to determine a possibly monoxenous life history of this parasite, suggested in the literature, two laboratory-raised chicks were each fed several hundred embryonated eggs just recovered from the uteri of gravid female worms;

in addition, two other chicks were fed several hundred eggs which had been kept for 1 week in water at room temperature (about 24° C). These four birds were killed 25 days after the feeding of the eggs and were found to be free of all helminths.

The following 5 species of insects collected in poultry farms have been found naturally infected with encysted larvae of *S. brumpti*: (Coleoptera) *Dermestes vulpinus, Gonocephalus seriatum, Ammophorus insularis, Alphitobius diaperinus;* and (Dermaptera) *Euborellia annulipes.* An examination of 20 specimens of insects collected in 1 poultry farm near Honolulu revealed the following incidence of infection: *A. diaperinus,* 75 per cent; *A. insularis,* 90 per cent; *E. annulipes,* 70 per cent. Encysted larvae of *S. brumpti* were also found in grasshoppers, *Conocephalus saltator,* 15 days after they were experimentally fed embryonated eggs of this parasite.

A knowledge of the life history of *Subulura brumpti* is not only of economic importance in order that control measures may be properly advocated, but it is also of zoological interest. Subulurids have been ascribed to the superfamily Ascaroidea. As far as the writer is aware, among the nematodes of vertebrates the only heteroxenous parasites reported to require arthropods as intermediate hosts belong to the superfamilies Filaroidea and Spiruroidea. In a few cases in which members of other superfamilies are heteroxenous, they have been reported as utilizing as intermediate hosts not arthropods but fish (as for certain members of the Ascaroidea, family Heterocheilidae) or earthworms and snails (as for certain members of the Strongyloidea, family Metastrongylidae). It is, therefore, of interest to find a member of the Ascaroidea to be heteroxenous and to utilize arthropods as intermediate hosts.

The writer acknowledges the assistance received by Dr. E. W. Price and Mr. J. T. Lucker, members of the Bureau of Animal Industry, Washington, D. C., for supplying information relative to any published reports on the life history of *S. brumpti.*—Joseph E. Alicata, *University of Hawaii, Honolulu, T. H.*

PRELIMINARY NOTE ON THE LIFE CYCLE OF *LEPTORHYNCHOIDES THECATUS,* AN ACANTHOCEPHALAN PARASITE OF FISH

During the past summer while working at the University of Michigan Biological Station under the direction of Dr. Lyell J. Thomas, the author was able, by feeding the eggs, to infect experimentally laboratory-reared amphipods, *Hyalella knickerbockeri,* with the larval stage of *Leptorhynchoides thecatus.*

The development of the larva within the amphipod was followed through to the 22nd day, at which time the amphipoda succumbed to the effects of the large number of acanthocephalan larvae developing within them.

It was found that, after being freed from the egg membranes within the amphipod intestine, the acanthocephalan embryos passed through the intestinal wall and became attached to the coelomatic side of the amphipod intestine. In this position the embryos developed into a round bladder-like structure.

After approximately sixteen days of attachment to the amphipod intestine, the larvae were found to have released their hold and were free within the body cavity of the amphipod. Once free within the body cavity, the larvae lost their bladder-like shape and became elongated and "S" shaped. This "S" shape is the typical appearance of the 22-day larvae. Within them could be seen a well developed proboscis sheath and a clearly outlined genital ligament containing within it the primordia of the future genital apparatus. As yet there were no visible proboscis hooklets.

With new experiments now under way the author will report on the complete life cycle of *Leptorhynchoides thecatus* in the very near future.—Dominic L. DeGiusti, *University of Michigan Biological Station and University of Wisconsin.*

THE IMMUNIZATION OF RATS OF DIFFERENT AGE GROUPS AGAINST *TRYPANOSOMA LEWISI* BY THE ADMINISTRATION OF SPECIFIC ANTISERUM PER OS

In a paper published in this journal (1938, J. Parasitol. **24**: 65) the present author reported that the young of a mother rat which is immune to *Trypanosoma lewisi* are also resistant to this parasite, the resistance being acquired by the young largely after birth through the ingestion of the milk of the immune mother. If the young of a perfectly normal mother be permitted to nurse upon the immune mother beginning at any time up to at least the 15th day after birth, they readily acquire resistance. The rather remarkable facility with which the young of all the nursing ages tested acquired immunity led to the experiment which follows to determine whether rats of different age groups could be protected against *T. lewisi* by the administration of a specific antiserum per os.

Rats of 10, 15, 20, 25, 40, and 60 days of age at the time of infection were used in the present experiment. These were given 0.25 cc of specific antiserum per 10 gm body weight by syringe through a blunted hypodermic needle lowered carefully into the esophagus one day before infection, the day of infection, and on four successive days thereafter. A 20 gauge needle 1.0 inch long was used for the animals 25 days old or less, and a 16 gauge needle 1.5 inches long was used for the older rats. The needles were blunted by removal of their beveled tips with a file. The rats were infected intraperitoneally with 1 million *T. lewisi* per 100 gm body weight, and were examined daily thereafter for infection. One control rat of each age group was given the proportionate dose of the same antiserum intraperitoneally on the day of infection only, and another control rat of each age

TABLE 1.—*Immunization of rats of different age groups against* Trypanosoma lewisi *by the administration of a specific antiserum per os*

Rat No.	Age (days) when infected	Weight (gms) when infected	Volume (cc) antiserum given each dose	Course of infection, on designated days						
				1	2	3	4	6	8	10
1	10	16.5	.41	0	0	0	0	0	0	0
2	10	17.0	.42	0	0	+	+	0	0	0
3	10	21.0	.52	+	+	0	+	0	0	0
4	10	14.5	.36	+	+	+	++	7T	224T	80T
5	10	14.5	None	+	26T	92T	580T	Dead		
6	15	21.0	.52	0	+	0	+	++	52T	17T
7	15	20.0	.50	0	0	+	+	53T	40T	17T
8	15	27.5	.68	0	+	+	12T	149T	33T	12T
9	15	29.0	.72	+	+	+	12T	156T	40T	23T
10	15	25.5	None	+	++	53T	220T	1155T	Dead	
11	20	31.5	.78	+	0	+	3T	42T	124T	14T
12	20	30.0	.75	+	+	68T	175T	325T	45T	2T
13	20	30.5	.76	0	++	50T	225T	628T	562T	Dead
14	20	31.0	None	+	++	64T	250T	563T	213T	81T
15	25	34.0	.85	0	++	40T	163T	183T	52T	10T
16	25	35.5	.88	+	++	42T	155T	80T	7T	1/5
17	25	35.0	None	+	++	22T	78T	75T	33T	20T
18	40	48	1.20	+	+	+	56T	66T	46T	46T
19	40	54	1.35	+	++	++	97T	142T	52T	11T
20	40	50	None	0	++	8T	75T	140T	63T	25T
21	60	116	2.90	+	++	21T	117T	163T	45T	8T
22	60	53	1.32	+	++	68T	240T	470T	350T	244T
23	60	105	None	+	++	43T	79T	38T	15T	1/3

One control rat of each age group was given the proportionate dose of antiserum intraperitoneally on the day of infection. All were completely protected.

Key to observations:

+ = less than 1 parasite per high-dry field of microscope (× 430).
++ = from 1 to 5 parasites per high-dry field of microscope.
5T, 30T, etc. = thousands of trypanosomes per cu mm tail blood by hemacytometer.
0 = no parasites in 50 microscope fields.

group was infected but given no serum whatsoever. The results for typical animals of this experiment are given in the accompanying table.

It is seen from the table that rats 20 days old or less when infected were protected by an antiserum given per os. In some cases among those 10 days old, this protection was complete, although usually a few trypanosomes could be observed after careful search. In some 10 day old rats, however (e.g., Rat No. 4), the resistance was lost as soon as the administration of serum was discontinued. Rats 15 days old when infected acquired some resistance after oral administration of antiserum, although in no case was the protection complete and the resistance was promptly lost after the last dose of serum. Rats 20 days old when infected also acquired some very slight transitory resistance after receiving antiserum orally. No evidence whatsoever was obtained that rats over 20 days old could be measurably protected in this manner, since those rats given serum developed infections of as great intensity as those seen in control rats given no serum.

It is concluded that rats can be immunized against *T. lewisi* by the oral administration of a specific antiserum throughout the usual nursing period (21 days), although late in this period with progressively less effectivity. Whether the intestine of older animals becomes impermeable to the antibody or develops the capacity to denaturize this antibody is as yet not disclosed.—JAMES T. CULBERTSON, *Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.*

TRANSMISSION OF RESISTANCE AGAINST *TRYPANOSOMA LEWISI* FROM A PASSIVELY IMMUNIZED MOTHER RAT TO YOUNG NURSING UPON HER

The young born of a mother rat immunized through recovery from an infection with *Trypanosoma lewisi* are also resistant to this parasite, the resistance being acquired by the young largely after birth through ingesting the milk of the immune mother (1938, J. Parasitol. **24**: 65). The present communication offers data to show that a normal mother rat passively immunized against *T. lewisi* also is able to transfer to her young resistance against the homologous parasite.

Two normal mother rats, numbers 225 and 227, were obtained *immediately* after delivering, respectively, 10 and 5 young. Three of the young of one mother were promptly exchanged with three of those of the alternate mother. Beginning one day after delivery, mother 225 was injected intraperitoneally daily for 5 days

TABLE 1.—*The transmission of resistance against* Trypanosoma lewisi *from a mother rat to the young nursing upon her*

Rat No.	Born of Rat No.	Nursed by Rat No.	Observation on designated days after infection								
			2	4	6	8	10	12	15	17	20
1 ...	225	225 (Passively immunized after delivery)	0	0	0	0	0	0	0	0	0
2 ...			0	0	0	0	0	0	0	0	0
3 ...			+	0	0	0	0	0	0	0	0
4 ...			+	0	0	0	0	0	0	0	0
5 ...			+	+	++	+	+	+	0	0	0
6 ...			+	+	+	+	0	0	0	0	0
7 ...			+	++	10T	400T	Dead				
8 ...	227		0	0	0	0	0	0	0	0	0
9 ...			0	0	0	0	0	0	0	0	0
10 ...			0	0	+	+	+	+	+	+	0
11 ...			++	40T	100T	279T	Dead				
12 ...			++	62T	60T	50T	124T	30T	+	+	0
13 ...	225	227 (Normal)	++	177T	325T	87T	24T	15T	++	+	0
14 ...			++	88T	175T	513T	Dead				
15* ..			0	0	0	0	0	0	0	0	0

Key to observation: 0 = no trypanosomes 50 microscope fields (×430)
+ = less than 1 trypanosome per field
++ = from 1 to 5 trypanosomes per field
40T, 100T = thousands of trypanosomes per cmm tail blood, by hemacytometer

* Injected intraperitoneally with 0.2 cc of the immune serum pool given Mother 225.

with 5 cc of the pooled serum from several rats bled within seven days after recovery from infection with *T. lewisi.* Mother 227 was injected similarly with normal rat serum. One of the young (No. 15), born of mother 225 but nursed by mother 227, was injected intraperitoneally with 0.2 cc of the pool of immune serum on the day of birth to test the potency of the immune serum being used. Three days after the first serum injections into the mother rats, all of the young rats were injected with 50,000 living *T. lewisi,* and were examined daily thereafter for infection. The results of the observations are given in Table 1.

Of the 10 young nursed by the passively immunized mother, 4 (2 own young and 2 of the alternate mother) proved completely resistant, and the remaining 6 showed some degree of resistance. One of the 6 animals, after an initial resistant interval became severely infected and died on the seventh day, but all others (with one exception, and that on a single day only) showed never more than 1 parasite per field. Of the 5 young nursed by mother 227, given normal serum, 2 (1 own young and 1 of mother 225) died and 2 suffered intense infections lasting 17 days. Young rat No. 15, used to test the potency of the immune serum employed, remained free of infection.

It is clearly shown by this experiment that a normal mother rat, passively immunized after delivery against *Trypanosoma lewisi* by injection intraperitoneally with the serum of a specifically immunized rat, will confer resistance to young rats nursing upon her. It is thus indicated that the resistance naturally acquired by young rats from an immune mother is dependent upon the transfer to the young of an antibody identical with or similar to that found in the blood of the parent.—JAMES T. CULBERTSON, *Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.*

A NEW ANTHELMINTIC "RAIGAN" FOR TAENIASIS

Over twenty different kinds of anthelmintics are mentioned in old Chinese literature, but according to our studies most of them have no effect on parasitic worms. However, we have found that one anthelmintic known as Raigan has a specific action on tapeworms. An article on Raigan was published by Dr. Ryo two years ago. We present in this paper a short account of our investigations.

Raigan is a sort of mushroom (*Omphalia lapidescens*) which grows on sandy soil where bamboo trees are luxuriant. It is dried to the hardness of stone and sold in the market. Raigan is mentioned in old Chinese literature published at the beginning of the Christian era. It was recognized as an anthelmintic, but it is not clear against which kinds of worms it was used. Dr. Ryo believes that *Ascaris, Oxyuris,* and *Taenia* were the only parasitic worms known in ancient times. Apparently Raigan was not applied specifically to taeniasis.

Our studies of Raigan were carried on with *Taenia saginata, Taenia solium, Hymenolepis diminuta* and *Hymenolepis nana* of man and *Dipylidium caninum* of dogs. We had no opportunity to experiment with *Diphyllobothrium.*

At first we treated patients with doses of 20 gr of powdered Raigan three times per day for three successive days. This treatment was successful in over a hundred cases of taeniasis. No preparatory treatment is necessary; patients do not need to remain in the hospital; and no unpleasant complications were encountered. After we had succeeded in extracting the active principle from the crude material, we reduced the doses from 20 gr to 0.4 gr. A complete cure was always obtained by giving three doses of 0.3 gr three times per day for three successive days. No purge should be employed. The worms are expelled in small segments during the period of about one week after the first dose is given. The drug apparently destroys the tissues of the worms. The young proglottides are expelled in small yellowish-brown pieces which do not exhibit any movement in warm saline solution. Old proglottides are sometimes expelled in longer sections. The scolex and neck portion of *T. saginata* and *T. solium* were never recovered. No reappearance of the worms took place within four months after treatment. Hence we believe the worms were destroyed. The scolex of *Hymenolepis* was never

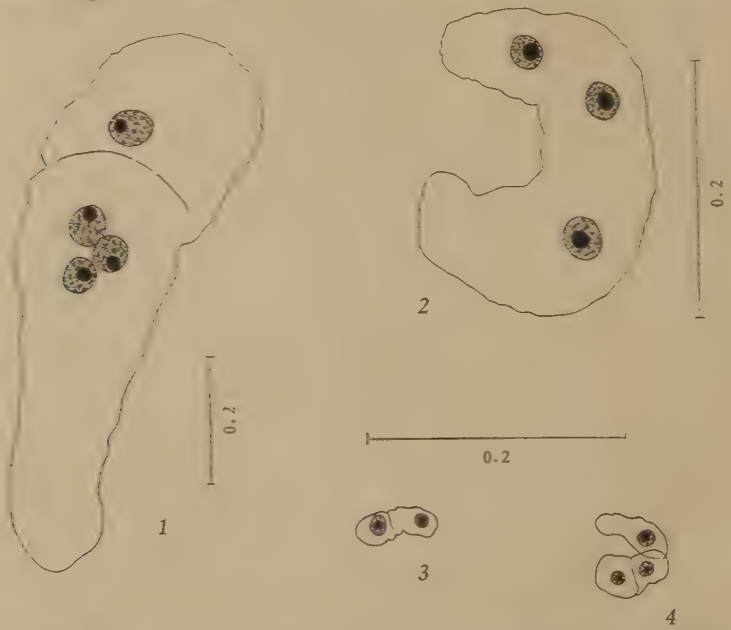
recovered, and the proglottids of this type of worm were very seldom seen. We assume that *Hymenolepis* was eliminated since eggs disappeared from the feces.

Raigan has no effect on *Ascaris, Ancylostoma,* etc. The chemical nature of the active principle of Raigan has not yet been determined.

We shall be glad to supply this drug to investigators in large amounts at cost or in small amounts gratis.—KENTARO HIYEDA AND BUNJIRO TERADA, *Manchuria Medical College, Mukden, Manchoukuo.*

GREGARINES FROM THE CRAB, *CANCER PRODUCTUS*

The examination of the intestinal contents of seven individuals of the crab, *Cancer productus,* revealed three infected with gregarines. The crabs were taken at Argyle Bay, San Juan Island, Washington, on August 1, 3, and 18, 1937. The gregarines seemed to be present only in the mid-gut and the anterior one-third of the hind-gut. They occurred in three size-ranges, as indicated by specimens measuring approximately 0.025 × 0.063 mm, 0.136 × 0.410 mm, and 0.300 × 0.870 mm. (Figs. 1, 2, 3, and 4.)



Figs. 1–4. Gregarines from the crab. Scale in millimeters. Fig. 1. Reconstructed from 8 serial sections of intestine. Fig. 2. Reconstructed from 11 serial sections of intestine. Fig. 3. Drawn from a single section of intestine. Fig. 4. Reconstructed from 3 serial sections of intestine.

Although the material examined was not adequate to permit identification or diagnosis, it seems to be of sufficient interest to warrant reporting at this time, since a check of the literature indicates that *Cancer pagurus* is the only crab of that genus reported as a host to a gregarine, an unnamed species of uncertain position (Kamm, 1922, Illinois Biol. Monogr. 7: 32).—ASHTON C. CUCKLER AND EDSON FICHTER, *Department of Zoology, University of Nebraska.*

THE VALIDITY OF *DIPYLIDIUM SEXCORONATUM* VON RÁTZ 1900 (CESTODA)

Witenberg (1932, Z. Parasitenk. **4**: 549) reduced *Dipylidium sexcoronatum* von Rátz 1900 to a synonym of *D. caninum* (Linnaeus 1758) Leuckart 1863. His reason for this was that although the descriptions give 6–7 rows of rostellar hooks to *D. sexcoronatum* and 3–4 rows to *D. caninum*, he had been unable to find a single specimen of this latter species with but 3 rows of hooks on the rostellum; instead he had found them to be armed with 4–7 rows.

Neveu-Lemaire (1936, Traité d'Helminthologie Médicale et Vétérinaire, Vigot Frères, Paris: 461) has recently followed the same practice upon the basis of Witenberg's paper and upon the statement of Baylis (1929, A Manual of Helminthology Medical and Veterinary, Baillière, Tindall and Cox, London: 99–100) that in his opinion it is impossible that all of the forms allocated to the genus *Dipylidium* can be maintained as distinct species and that "many of them may represent nothing more than individual or local variations within a much smaller number of genuine species." Baylis further stated that *D. caninum* is extremely variable and that the difficulty of accurately counting the rows of hooks on the rostellum has probably influenced the number of supposed species in this genus. He also pointed out that these hooks are not arranged in straight transverse rows, as they are usually described, but appear to be in longitudinal rows tending to run spirally around the rostellum. However, Baylis did not list *D. sexcoronatum* as a synonym of *D. caninum* but distinguished the two species in the usual way, *viz.*:

D. caninum	*D. sexcoronatum*
Strobila 15–50 cm long	Strobila 10–30 cm long
Maximum width of strobila 2–3 mm	Maximum width of strobila 2 mm
3–4 crowns of hooks on rostellum	6–7 crowns of hooks on rostellum
Hooks 12–15 μ long anteriorly	Hooks 11 μ long anteriorly
" 5–7 μ " posteriorly	" 4 μ " posteriorly
100–200 testes in each mature segment	130–175 testes in each mature segment
Cirrus sac usually not reaching lateral excretory canals	Cirrus sac extending as far as but not beyond lateral excretory canals
4–20 eggs in each egg capsule	2–15 eggs in each egg capsule

The fundamental differences between species of the same genus are physiological and not necessarily morphological. Despite the overlapping of characters shown above, physiological differences between these two cestodes have been demonstrated. Zimmermann (1937, Z. Parasitenk. **9**: 717–729) confirmed previous investigators' statements that *Ctenocephalides felis* (Bouché) and *C. canis* (Curtis) are intermediate hosts of *Dipylidium caninum*, but she was entirely unable to infect *Trichodectes canis* Degeer. She determined that *T. canis* is the intermediate host for *D. sexcoronatum* and that differences occur between the cysticercoids in fleas and those in lice, which is a manifestation of a difference in the species. The work of Zimmermann indicates that Melnikoff (1869, Arch. Naturg. **25**: 62–69) was working with *D. sexcoronatum* rather than with *D. caninum* when he described *T. canis* (= *T. latus* Nitzsch) as the intermediate host of the latter tapeworm. The present writer (1937, Cornell Vet. **27**: 338–348) has shown that iso-amyl-ortho-cresol is a highly efficient anthelmintic in the treatment of *D. caninum* infections in dogs, but when administered at the same dosage rate to dogs infected with *D. sexcoronatum* no elimination is effected. Inasmuch as a biochemical reaction is involved here and the variable in the two instances is the parasite, the differences in the action of the drug upon the two worms clearly indicate a difference in physiology. This in turn indicates that the two species are distinct. It should not be inferred, however, that the ability of a drug to remove some worms and not others is necessarily a criterion for species differentiation. Such a phenomenon is only a partial criterion and should be correlated with other criteria, morphological and bionomic, and is applicable only where a markedly specific drug, such as iso-amyl-ortho-cresol, and not a less specific drug, such as carbon tetrachloride or tetrachlorethylene, is used.

In the opinion of the writer the above mentioned data demonstrate clearly the validity of *Dipylidium sexcoronatum* as a species and not a synonym of *D. caninum*.—M. A. Stewart, *Division of Entomology and Parasitology, University of California, Davis, California.*

AN ANOPHELINE FROM INLAND GEORGIA RESEMBLING THE BRACKISH-WATER RACE OF *ANOPHELES CRUCIANS*

During October, 1937, anopheline larvae with greatly reduced palmate hairs on abdominal segments 3 and 7, and 1- to 4-branched antepalmate hairs (hair no. 2) on abdominal segments 3, 4, and 5, were collected in inland south Georgia counties. Reference was made to Bradley's (1936, South. Med. J. **29**: 857–862) key to the anopheline larvae of the United States, and the specimens were tentatively identified as larvae of the brackish-water race of *Anopheles crucians*. They were first noted in collections from Sumter County, roughly 150 miles from the nearest coast line, and shortly thereafter in Brooks County about 50 miles inland. During the winter of 1937–38, specimens were taken in Brooks, Sumter, Terrell, and Thomas Counties, Georgia; the latter two counties are about 125 and 50 miles inland, respectively.

Chemical analysis of a water sample from a site in Brooks County where numbers of the larvae had been collected revealed that the chloride content was no more than that of inland waters in general.

No obvious characters were observed which could be used to distinguish the reared adults of this form from those of typical fresh-water *Anopheles crucians*. Eggs were not observed nor have the hypopygia of the reared males been studied. Specimens of the larvae were compared to two slide mounts of larvae of the brackish-water race of *Anopheles crucians*. The palmate hairs on abdominal segments 3 and 7 were more markedly reduced on the larvae of the inland form than they were on those segments of the brackish-water race, while branching of the antepalmate hairs on segments 3, 4, and 5 was better developed in the inland form.

Larval specimens of this anopheline have been collected at 6 situations in Sumter County, 6 in Thomas County, 2 in Terrell County, and 1 in Brooks County. The accumulation of observations on these 15 breeding places points to pastureland seepage areas (helocrene springs) with acid waters as the typical habitat. Upwards of 25 specimens of larvae of this anopheline were identified in each of several collections from situations of this type (pH readings 5.0, 5.3, and 5.8 by Hellige colorator); other anophelines were not encountered in these collections. However, on subsequent visits to some of these situations smaller numbers of the larvae of typical freshwater *A. crucians* and *A. punctipennis* were found in association with the above-mentioned larvae.

A few specimens of the form have been collected in swampy, sluggish streams, but in this type of situation have always been outnumbered by larvae of the typical freshwater *A. crucians*. Once a single larval specimen of the form was taken in a shallow pond (pH 6.6) in association with larvae of *A. walkeri* and *A. crucians*, and from a similar situation (pH 7.2) a specimen was taken with larvae of *A. punctipennis, A. walkeri,* and *A. crucians*.

The taxonomic status of the form is being studied by Dr. W. V. King, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.—R. Edward Bellamy, *Division of Malaria Investigations, Georgia Department of Public Health, Atlanta, Ga.*

DIASIA PODILYMBI, CORRECTION FOR *DIASIA PODILYMBAE* OLSEN, 1938

For a new species of trematode belonging to the genus *Diasia* from the pied-billed grebe, *Podilymbus podiceps* Olsen (1938, J. Parasitol. **24**: 215–218) proposed the name *D. podilymbae*. Since *Podilymbus* is masculine, the genitive should be *podilymbi* instead of *podilymbae* as originally designated.—O. Wilford Olsen, *University of Minnesota, St. Paul.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Fourteenth Annual Meeting, Richmond, Virginia, December 28, 29, and 30, 1938*

Minutes of Fourteenth Annual Business Meeting

The Fourteenth Annual Business Meeting of the Society was held at Hotel Richmond, Richmond, Virginia, on December 29, 1938, following the annual Parasitologists' Luncheon. One hundred and fifteen members and guests were present. The meeting was called to order at 1:35 PM by President F. C. Bishopp. The minutes of the 27th Council Meeting held at Hotel Richmond on December 27, 1938, were read by the Secretary. It was noted that the Council had unanimously elected Professor Lauro Travassos, Instituto Oswaldo Cruz, Rio de Janeiro, and Dr. C. M. Wenyon, Wellcome Research Institution, London, honorary foreign members of the Society. Upon motion the minutes were approved as read.

I. Reports of Officers

1. The report of the Secretary was presented. It was noted that in accordance with the motion passed at the last annual meeting a referendum of the Society regarding an annual $2.00 assessment for the support of Biological Abstracts had been conducted and that the Council in a mail ballot had voted against such a proposal. As of December 22nd there were 390 active members on the roll of the Society, 18 new members having been elected during the course of the year. Subsequent to this date, 28 additional new members were elected by the Council. Upon motion the report was accepted and placed on file. Upon the suggestion of the Chair, Dr. G. W. Hunter, III, made some announcements concerning the present plan of publication of Biological Abstracts, and suggested ways that the Society could cooperate through the Biological Abstracts Committee authorized at the 27th Council Meeting.

2. The report of the Treasurer, copy attached, certified as correct by the auditing committee of the Council, was accepted, and placed on file.

II. Reports of Committees

1. The report of the status of the Secretarial Fund was presented by the Custodian, Dr. Norman R. Stoll. Upon motion the report was accepted and an audited copy was placed on file.

2. The report of the Committee on Necrology was read by the Secretary. The deaths of four members since the last annual meeting were reported:

W. T. Johnson, of Oregon Agricultural College, on December 29, 1937.

M. C. Hall, of the National Institute of Health, on May 1, 1938.

H. B. Raffensperger, of the Bureau of Animal Industry, U. S. Department of Agriculture, on May 22, 1938.

E. B. McKinley, of George Washington University, on July 29, 1938.

Members of the Society stood for a moment in silence as a tribute of respect to the memory of these men.

3. The report of the Editorial Committee was presented by Dr. Norman R. Stoll. Papers are now being accepted for the June number of the Journal. The interval between first receipt from author and publication has averaged 8.8 months for regular articles, 7.7 months for research notes published during the past year (volume 24). Upon motion the report was accepted with a vote of thanks to the Committee for their efficient work in publishing the Journal.

*Abstracts of papers presented were published before the meetings in the Journal of Parasitology, Dec. Suppl., 1938. A general report of the program appeared in *Science*, Feb. 3, 1939 (89: 101).

III. Reports of Representatives of the Society

1. The report of the representatives on the Council of the American Association for the Advancement of Science was given by Dr. Ernest C. Faust. Henceforth members of the Association who are in arrears for more than one year will be dropped from membership. Upon motion the report was accepted.

2. The report of the representatives on the Council of the Union of American Biological Societies was presented by Dr. G. L. Graham. The present status of Biological Abstracts and the plans for future publication were reported, and, also, the progress of the Committee of the Union of American Biological Societies dealing with the teaching of biology. Upon motion the report was accepted.

IV. New Business

Upon a motion in accordance with the recommendation of the Council, Colonel Charles F. Craig was unanimously elected a life member of the Society.

The Secretary presented the Council nominations for officers of the Society for 1939 as follows: President, for one year, Horace W. Stunkard; Vice-president, for one year, H. E. Meleney; Treasurer, for two years, G. F. Otto; Members of the Council, for four years (to succeed J. E. Ackert and W. W. Cort), E. R. Becker and E. W. Price; Members of the Editorial Board, for four years (to succeed A. C. Chandler, C. A. Kofoid and Robert Matheson), A. C. Chandler, E. E. Tyzzer and Cornelius B. Philip. There were no further nominations and upon motion, duly seconded, the Secretary was instructed to cast one ballot for the nominations as presented.

A vote of thanks was extended to Dr. Bruce D. Reynolds for his services as local representative in helping to make arrangements for the Richmond meeting.

At 2:35 PM it was voted that the Society adjourn to convene again in December, 1939, at Columbus, Ohio, in conjunction with the meeting of the American Association for the Advancement of Science.

Respectfully submitted,

O. R. McCoy, *Secretary*